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CRYOPRESERVATION OF RED CELLS: ACHIEVEMENTS AND PROSPECTIVE,(U)

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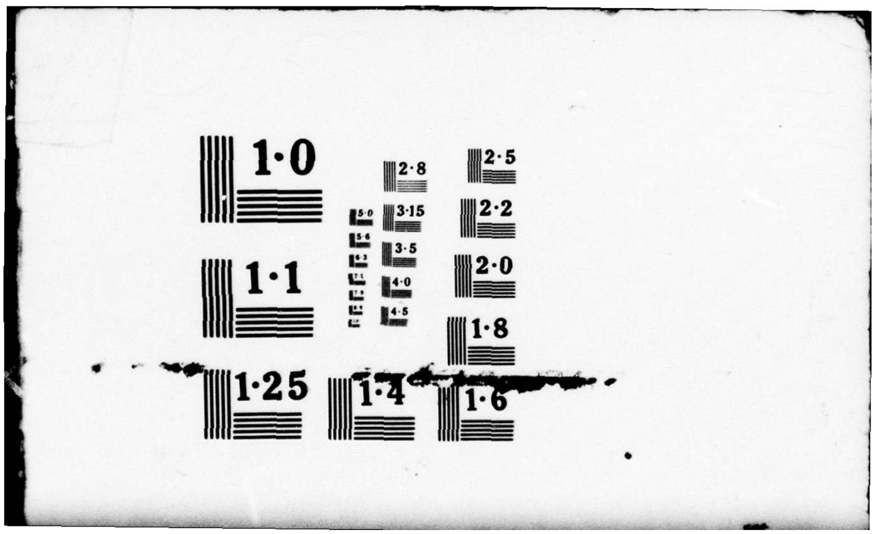
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Preserved red cells are transfused to increase the delivery of oxygen to tissue. It is also essential that the preserved red cells circulate in order to increase the red cell mass and to improve the oxygen carrying capacity. The delivery of oxygen to tissue immediately after transfusion of preserved red cells depends to a great extent on their affinity for oxygen. Red cells stored in ACD for 7 days at 4 C maintain their ability to carry oxygen, but their ability to release it is impaired. It was not until 1967 that the correlation between oxygen

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transport function and the red cell 2,3 DPG level was appreciated. More recently investigators have realized the importance of the oxygen delivering capacity or tranfused red cells during the first 4 hours after transfusion. Red cells that have low 2,3 DPG levels and increased affinity for oxygen will increase the cardiac output and/or decrease the venous PO2 for 4 hours after transfusion.

CPD-preserved red cells are more likely to have normal oxygen transport than are ACD-preserved red cells. During storage at 4 C the oxygen transport function of CPD red cells is maintained by purine nucleoside supplementation and during freeze-preservation it is maintained with glycerol. By modifying the red cell biochemistry before freeze-preservation, it is possible to prepare viable red cells with 2,3 DPG levels that are 1-1/2 to 2 times normal and have decreased affinity for oxygen. Such red cells have acceptable posttransfuions survival and greater oxygen-releasing capacity for at least 72 hours after transfusion.

The well-being of certain patients may be placed in jeopardy if they are given preserved red cells that have increased affinity for oxygen, since the patient may not be able to meet the accompanying demand for increased blood flow, and the venous oxygen tension may fall to a critical level. Clearly, patients in hemorrhagic and septic shock, those subjected to extracorporeal circulation during cardiac surgery, and anemic patients with myocardial or cerebrovascular insufficiency can be handled most efficaciously by treating them with red cells that have 2,3 DPG levels that are either normal or about twice normal.

CRYOPRESERVATION OF RED CELLS:

ACHIEVEMENTS AND PROSPECTIVE

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The opinions or assertions contained herein are those of the author, and are not to be construed as official or reflecting the views of the Navy Department or Naval Service at large.

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For the past 10 years the Naval Blood Research Laboratory, Chelsea, Massachusetts, has been involved in extensive research into freeze-preservation of red blood cells. Glycerol has been added to concentrated red cells to achieve a final concentration of 20% W/V or 40% W/V. The red cells have been freeze-preserved, and after thawing have been washed to reduce the glycerol concentration to less than 1% W/V (Valeri, 1970). This procedure preserves only the red cells, and not the whole blood. Whole blood that is not used while it is fresh should be separated into its components, and the isolated red cell concentrates should be preserved by liquid and freezing procedures.

Acid-citrate-dextrose-(ACD) collected red cells can be kept at 4 C for no longer than 24 hours before freeze-preservation if the oxygen transport function is to be maintained. Citrate-phosphate-dextrose-(CPD) collected red cells can be kept at 4 C for 3 to 5 days. CPD maintains the oxygen transport function more adequately during pre-freeze storage than does ACD (Valeri, 1971; Valeri, 1974a); usually this function is not altered by freezing, thawing, or washing.

Red cell concentrates that were stored in CPD at 4 C for 3 to 5 days before freeze-preservation are referred to as <u>non-rejuvenated frozen red cells</u> (Valeri, 1974a). A simple procedure developed in our laboratory allows us to modify the biochemistry of red cells to control the oxygen transport function: this is called rejuvenation. Red cells that were stored in CPD at 4 C for 3 to 5 days and rejuvenated with a

solution containing pyruvate, inosine, glucose, phosphate, and adenine (PIGPA, Solution A) before freeze-preservation are referred to as <u>indated-rejuvenated frozen red cells</u> (Valeri, 1974b). Red cells that were stored in ACD or CPD at 4 C for as long as 35 days and rejuvenated with the PIGPA (Solution A or Solution B) before freeze-preservation are referred to as <u>outdated-rejuvenated frozen red cells</u> (Valeri and Zaroulis, 1972a,b). The additives used in the rejuvenation procedure are potentially toxic, but since they are removed during postthaw washing, they present no cause for concern. Like non-rejuvenated red cells, the rejuvenated red cells can be freeze-preserved with 20% W/V glycerol and storage at -150 C using liquid nitrogen, or with 40% W/V glycerol and storage at -80 C using mechanical refrigeration. The washed red cells are therapeutically effective and can be stored at 4 C in a sodium chloride-glucose-phosphate solution for at least 3 days before transfusion without deterioration (Figure 1) (Valeri and Zaroulis, 1972a,b; Valeri, 1974a). <u>FIG. 1</u>

This paper reports on the glycerolization and freeze-preservation of non-rejuvenated and rejuvenated red cells. The glycerol is removed by washing the thawed red cells with sodium chloride solutions in any one of three commercially available systems.

MATERIALS AND METHODS

Red Cell Collection and Storage At 4 C Prior to Glycerolization and Freeze-Preservation

From each healthy volunteer approximately 450 ml of blood was collected in a double or triple blood pack plastic bag[a] containing 63 ml of citrate-phosphate-dextrose (CPD) or 67.5 ml of acid-citrate-dextrose (ACD). The blood was centrifuged at 4500 X g for 3 minutes at 22 C in a Sorval RC-3 centrifuge,[b] and the platelet-rich plasma was expressed into a transfer pack. Platelet-poor plasma and cryoprecipitate were prepared, and platelet concentrates were prepared for liquid and freeze-preservation. The blood was kept at room temperature for as long as 4 hours while the components were being prepared. Some of the red cells were concentrated to hematocrits of about 70 V% and were stored in the liquid state for 3 to 5 days at 4 C. Units were prepared for glycerolization by centrifugation at room temperature (22 C) at 3000 X g for 7 minutes in a PR-6 centrifuge;[c] all the visible plasma was removed to obtain red cell concentrates with hematocrits of about 90 V%.

Still other units were stored at 4 C for as little as 3 days or as long as 35 days before incubation at 37 C for 1 hour with a rejuvenation solution (vide infra) prior to glycerolization and freezing. Rejuvenation was performed in the following manner: After the concentrated red blood cells had been stored at

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[a] Fenwal Laboratories, Morton Grove, Ill.

[b] Ivan Sorvall Co., Newtown, Conn.

[c] International Equipment Co., Needham, Mass.

4 C in CPD for 3 to 5 days (indated red cells) or in ACD or CPD at 4 C for 22 to 35 days (outdated red cells), a 50 ml volume of a rejuvenation solution was added to each unit. The rejuvenation Solutions A and B containing pyruvate, inosine, glucose, phosphate, and adenine are described in Table 1. TABLE 1 Using an AE-7 connector,[a] a 50 ml aliquot of the rejuvenation solution was added aseptically to the red cell concentrates of 70 V%. Each unit of blood was then placed in a water-tight plastic envelope and incubated at 37 C with agitation for 1 hour. The red cells were concentrated by centrifugation, the supernatant fluid was removed, and the glycerol was added to the red cell concentrates with hematocrits of 90 V%.

High Glycerol Red Cells

Red cell concentrates were placed in a modified Eberbach shaker[d] (2-speed power unit) capable of holding 8 units of concentrated red cells in a horizontal position. Each plastic bag was secured to prevent slippage during agitation. With an AE-7Y connector set,[a] a volume of the 6.2 M glycerol solution containing per 100 ml: 57.1 g of glycerol, 0.03 g of potassium chloride, 0.04 g of magnesium chloride, 1.6 g of sodium lactate, and 0.08 g of disodium phosphate, adjusted to pH 6.8, was added to the concentrated red cells. Table 2 TABLE 2 shows the initial and total volume of the 6.2 M glycerol solution that was required to achieve a final glycerol concentration of about 40% W/V. The non-rejuvenated red cell concentrates were stored for about 2 hours at room temperature

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[d]Cryogenic Equipment Corp., Buckeystown, Md.

prior to glycerolization. The rejuvenated red cell concentrates were kept for about 2 hours at room temperature, and then for 1 hour at 37 C prior to glycerolization. The glycerol solution either was kept at room temperature (22 to 25 C) or was warmed to 37 C prior to use. The initial volume of glycerol solution was added to each red cell concentrate in about 2 minutes using the modified shaker set at a low speed (about 150-200 cycles per minute). The glycerol-red cell mixture was stored at room temperature for about 10 minutes, during which time it was transferred to a bioriented polyolefin plastic bag.[e] After 10 minutes the remainder of the glycerol solution (Table 2) was added to the red cell-glycerol mixture with manual agitation. The plastic bag was placed in a special freezing frame[d] and stored in a -80 C mechanical freezer.

Low Glycerol Red Cells

Using the modified shaker, red cells were glycerolized to a final concentration of about 20% W/V by the addition of a solution containing per 100 ml: 35.0 g of glycerol, 2.88 g of mannitol, and 0.65 g of sodium chloride, equal to the weight of the red cell concentrate with a hematocrit of about 90 V%. The non-rejuvenated red cell concentrates were stored for about 2 hours at room temperature prior to glycerolization. The rejuvenated red cell concentrates were stored for about 2 hours at room temperature and then for 1 hour at 37 C prior to glycerolization. The glycerol solution either was kept at room temperature or was warmed to 37 C prior to use. The

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[e]UCAR, Union Carbide Corp., Chicago, Ill.

glycerol was added with lateral agitation of about 150-200 cycles per minute, and the red cells were placed in a bioriented polyolefin plastic bag[e] and stored in either aluminum or anodized containers. After rapid freezing by direct immersion in liquid nitrogen (-197 C), they were stored in the gas phase of liquid nitrogen at -150 C.

Thawing, Washing, and Postthaw Storage at 4 C

The 40% W/V glycerolized freeze-preserved red cells were thawed within 10 minutes at 37 C with mechanical agitation of the water. The thawed red cells were washed in one of the following ways: (1) by continuous-flow washing in the non-automated Haemonetics Blood Processor 15[f] using disposable rigid polycarbonate bowls and a bypass harness to connect the wash solutions to the washing bowl; (2) by continuous-flow washing in the automated Fenwal Elutramatic System[a]; or (3) by automated serial centrifugation in the IBM Blood Processor.[g] All of these washing systems utilize sodium chloride solutions. The 40% W/V glycerolized red cells were washed with the following solutions: 150 ml of 12 g% sodium chloride solution buffered with 25 mEq/liter of sodium phosphate to a pH of about 7.0; 1 or 2 liters of 1.6 g% sodium chloride solution buffered with 25 mEq/liter of sodium phosphate to a pH of about 7.0; and 1 liter of 0.9 g% sodium chloride solution containing 0.2 g% glucose buffered with 0.065 g% disodium phosphate to a pH of about 6.8.

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[f]Haemonetics Corp., Natick, Mass.

[g]IBM Corp., Princeton, N. J.

The 20% W/V glycerolized freeze-preserved red cells were thawed within 6 minutes at 42 C with manual agitation of the unit of blood in the water bath, and were washed as described above. These low glycerol red cells were washed with the following solutions: 500 ml of 3.2 g% sodium chloride solution buffered to a pH of about 7.0 with 25 mEq/liter of sodium phosphate, and 1 or 2 liters of 0.9 g% sodium chloride solution containing 0.2 g% glucose buffered with 0.065 g% disodium phosphate to a pH of about 6.8.

Haemonetics Blood Processor 15

This continuous-flow centrifugation system was not used with a programmer. The wash solutions were delivered by gravity flow into a disposable rigid polycarbonate bowl. It takes about 3 minutes to set up the bowl, the bypass harness, and the solutions. One hanger was 49-3/4" from the base of the pole, another was 32-1/4" from the base, and the third was 17-1/2" from the base. The topmost hanger held the thawed blood, the middle hanger held the 150 ml of 12 g% sodium chloride solution and 2 liters of 1.6 g% sodium chloride, and the bottom hanger held the 1 liter of 0.9 g% sodium chloride-glucose-phosphate solution. After thawing the high glycerol red cells were diluted with 150 ml of 12 g% sodium chloride solution and equilibrated at room temperature for at least 2 minutes. Using the bypass harness, these diluted red cells were delivered simultaneously with 2 liters of 1.6 g% sodium chloride solution into the disposable bowl which was spun at 4800 rpm at room temperature, followed by the addition of

1 liter of sodium chloride-glucose-phosphate solution. The flow rate of the diluted blood and the 3.2 liters of sodium chloride solution was approximately 200 ml per minute. Washing was performed at room temperature in about 20 minutes. The washed red cells had hematocrit values of about 40 V%, and the final wash solution served as the resuspension medium in which to store the washed red cells at 4 C for at least 24 hours before transfusion.

The thawed red cells containing 20% W/V glycerol were diluted with 500 ml of 3.2 g% sodium chloride solution. The diluted red cells were added to the spinning disposable polycarbonate plastic bowl, and when the supernatant fluid was displaced into the waste receptable, the residual diluted blood was added together with 2 liters of 0.9 g% sodium chloride solution containing 200 mg% glucose and 0.065 g% disodium phosphate buffered to a pH of about 6.8. The red cells were washed with 2.5 liters of wash solution by gravity flow in a disposable polycarbonate rigid bowl which was spun at 4800 rpm for about 15 minutes at room temperature. The flow rate was about 200 ml per minute. After washing, the red cells had hematocrits of about 40 V% and were stored in a sodium chloride-glucose-phosphate solution for at least 24 hours.

Only two units of high or low glycerol red cells of the same ABO and Rh blood type were washed in each 400 ml volume disposable bowl. Before transfusion the red cells were concentrated by centrifugation and the hematocrit was adjusted to about 90 V% by removal of all the visible supernatant solution.

Fenwal Elutramatic System

Both high and low glycerol red cells can be washed in this system. Two units of either 40% W/V or 20% W/V glycerolized red cells can be washed at one time using disposable polyvinyl chloride collapsible plastic bags. A programmer module was attached to an RC-3 Sorval centrifuge, and the diluted glycerolized red cells and the wash solutions were delivered into the washing bags. It takes about 10 minutes to set up the elutrapack[a] and the solutions in the washer. The elutrapack was used with 2 Y-sets. Prior to connection to the elutrapack, the 40% W/V glycerolized red cells were diluted with 150 ml of 12 g% sodium chloride solution and equilibrated at room temperature for at least 2 minutes. They were then diluted with 500 ml of 1.6 g% sodium chloride solution. The diluted red cells were connected to one segment of the Y-set, and 1 liter of the 1.6 g% sodium chloride solution was connected to the other. By pulsating flow of about 500 ml per minute, the diluted blood was delivered into the collapsible polycarbonate bags whose volume was about 600 ml. The spillage of the red cells from both bags started the centrifuge to accelerate to a speed of 3100 rpm at a temperature of 22 to 25 C. The centrifuge spun for about 20 seconds with no flow of the fluid through the seal, during which time the centrifuge speed accelerated from 0 to 1600 rpm. The fluid was then pumped through the rotating seal. After sedimentation of the red cells within the washing bag, pulsatile flow was resumed and the remainder of the diluted glycerolized red cells was transferred into the washing bag together with the 1.6 g% sodium chloride

solution at a flow rate of about 110 ml per minute. The diluted red cells and the 1.6 g% sodium chloride solution were delivered, and by setting the timer for 12 minutes all of the red cells were recovered. The containers of diluted blood and of 1.6 g% sodium chloride solutions were adjusted to a height that would permit simultaneous delivery of all of the diluted blood and about 200-300 ml of 1.6 g% sodium chloride solution within 9 minutes, and then delivery of 250-300 ml of 1.6 g% sodium chloride solution within 9 minutes, and then delivery of 250-300 ml of 1.6 g% sodium chloride solution into the washing bag within the final 3 minutes. After all of the red cells and most of the 1.6 g% sodium chloride solution had been added, 1 liter of 0.9 g% sodium chloride-glucose-phosphate solution was added by switching to the second timer for an 8-minute period. Two units of red cells containing 40% W/V glycerol were washed at the same time; each unit required a total of about 2.7 liters of sodium chloride solution. The washed red cells can be concentrated by centrifugation at 3100 rpm for 2 minutes, and after the supernatant fluid is removed, the concentrated red cells are transferred into the administration bag. Alternatively, the washed red cells with hematocrits of about 40 V% can be transferred at a flow rate of 500 ml per minute into the administration bag and stored at 4 C for at least 24 hours, at which time they can be centrifuged, the supernatant fluid removed, and the hematocrit value adjusted to about 90 V%.

The thawed 20% W/V glycerolized red cells were washed with 750 ml of 3.2 g% sodium chloride solution and 1 liter of sodium

chloride-glucose-phosphate solution. First they were diluted with 250 ml of 3.2 g% sodium chloride solution, after which the diluted red cells and 500 ml of the 3.2 g% sodium chloride solution were connected by a Y-set to the elutrapack washing system. The diluted red cells were pumped at a flow rate of about 500 ml per minute into the washing bags, and when the bags were full the red cells spilled through the effluent tubes and triggered the centrifuge to spin while the pump was stopped. The centrifuge spun for about 20 seconds and the red cells were sedimented. The pulsatile flow was resumed, and the residual diluted blood together with the 500 ml of 3.2 g% sodium chloride solution were pumped into the washing bag at a flow rate of about 110 ml per minute. With the timer set at 5 minutes, all of the diluted red cells and most of the sodium chloride solution was delivered into the washing bag. One liter of 0.9 g% sodium chloride solution containing 200 mg% glucose and 0.065 g% disodium phosphate buffered to pH 6.8 was then added by switching to the second timer that controlled the pulsatile pump for 8 minutes. The washed red cells can be concentrated by centrifugation at 3100 rpm for 2 minutes, and after the supernatant fluid is removed, the concentrated red cells are transferred into the administration bag at a flow rate of about 500 ml per minute. Alternatively, the washed red cells with hematocrits of about 40 V% can be transferred at a flow rate of about 500 ml per minute into the administration bag and stored at 4 C for at least 24 hours, and before transfusion concentrated to hematocrits of about 90 V%.

IBM Blood Processor

Both high and low glycerol red cells can be washed in the IBM Blood Processor using automated serial centrifugation. It takes about 5 minutes to set up the washing harness and the wash solutions. The 40% W/V glycerolized red cells were diluted with 150 ml of 12 g% sodium chloride solution and equilibrated at room temperature for at least 2 minutes, and then diluted with 500 ml of 1.6 g% sodium chloride solution. With the programmer, the centrifuge speed was set at 3000 rpm. Approximately one-half of the volume of diluted red cells was added to the washing bag, and the red cells were sedimented for 2-1/2 minutes. When the centrifuge stopped, the supernatant was decanted. The rate of supernatant decantation was set at 350 ml per minute, the rate of pump restoration at 350 ml per minute, and the supernatant volume was adjusted to 600 ml. With to-and-fro agitation the remainder of the diluted red cells was added to the 650 ml volume polyvinyl chloride plastic washing bag. The centrifuge was spun at 3000 rpm for 2-1/2 minutes, and when it stopped the supernatant was decanted. With to-and-fro agitation 500 ml of 1.6 g% sodium chloride solution was added together with the residual diluted blood when present. The centrifuge was spun at 3000 rpm for 1-3/4 minutes, and when it stopped the supernatant was decanted. After this approximately 500 ml of 0.9 g% sodium chloride solution was added with agitation, and the supernatant was decanted on two separate occasions. The final hematocrit of the washed unit was adjusted to about 40 V%. After washing, which took about 20 minutes, the red cells were transferred into a transfer pack and

stored at 4 C for at least 24 hours. At the time of transfusion they were concentrated by centrifugation, the supernatant fluid was removed, and the hematocrit was adjusted to about 90 V%.

The same setting on the programmer was used to wash the low glycerol red cells. The thawed red cells were diluted with 500 ml of 3.2 g% sodium chloride solution, and were added to the washing bag in two parts. They were washed on two separate occasions with 500 ml of 0.9 g% sodium chloride-glucose-phosphate solution.

Bacteriologic Studies

Cultures of 0.5 ml aliquots were made on blood agar and in peptone broth before glycerolization, after thawing and washing, and after storage at 4 C for up to 7 days; these were incubated at 37 C for at least one week.

Measurements in Vitro of Washed, Freeze-Preserved Red Cells

Recovery *in vitro* (%) of the freeze-preserved red cells was measured after thawing (%), and after washing (%) (Valeri et al, 1970a). Supernatant hemoglobin concentrations (mg per 100 ml) and total amounts of supernatant hemoglobin (mg per unit) were measured upon thawing and after washing and during postthaw storage at 4 C as previously described (Valeri et al, 1970a). Osmolality of the resuspension medium was measured in milliosmoles per kg of water in an osmometer.[h] The amount of ^{125}I albumin that was removed during washing was measured. Red cell potassium and extracellular potassium were measured as previously described (Valeri et al, 1970a).

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[h] Advanced Instruments, Inc., Needham, Mass.

Biochemical Measurements and Oxyhemoglobin Dissociation Curve

Red cell 2,3 DPG and ATP levels were determined as previously described. Whole blood lactate was measured spectrophotometrically (Valeri and Fortier, 1969). Uric acid, phosphorus, and creatinine levels were determined in the AutoAnalyzer (Kraml, 1966; Hawks et al, 1954), and inosine and hypoxanthine levels by a modification of the enzymatic method of Kalckar (1947). Glycerol was also measured in the AutoAnalyzer by a chromotropic acid procedure both after thawing and after washing (Runck and Valeri, 1972).

The oxyhemoglobin dissociation curve was determined at 37 C by the Bellingham and Huehns procedure (1969) with the use of a diluted washed red cell suspension (1 volume of red cells to 70 volumes of 0.9 g per 100 ml sodium chloride buffered to pH 7.2 with 0.100 g per 100 ml disodium phosphate), and the P_{50} value is reported. The carboxyhemoglobin level was measured spectrophotometrically.[i]

Blood pH was measured at 22 C and at 37 C in a pH/gas analyzer. Red cell pH was measured at 37 C by the procedure of Hilpert and associates (1963).

Survival In Vivo of Rejuvenated and Non-Rejuvenated Freeze-Preserved Red Cells

The recipients were patients who required treatment of deficits in their red cell masses caused by traumatic injuries,

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[i]Co-oximeter, Model 182, Instrumentation Laboratories, Lexington,Mass.

neoplastic disorders, or other diseases. Some received as few as 2 and others as many as 6 units of either rejuvenated or non-rejuvenated freeze-preserved washed red cells. The compatibility of donor red cells was tested by saline, albumin, and anti-globulin procedures.

The recipient's red cell mass was measured immediately before transfusion with the use of 5 or 10 uCi of labeled sodium chromate ($Na_2{}^{51}CrO_4$) as described previously (Valeri et al, 1970a). A sample of the recipient's blood was collected before transfusion for measurement in the AutoAnalyzer as a guide in estimating the percentage of 'unremoved' recipient red cells in the circulation. Subsequent samples were collected immediately after the transfusion, 4 hours after transfusion, and 1, 2, and 3 days later, and in some cases at weekly intervals thereafter. The percentage survival of the donor red cells was determined using an automated differential agglutination procedure. Survival values of small aliquots of the rejuvenated and non-rejuvenated red cells were measured using a ^{51}Cr labeling procedure as previously described (Valeri, 1974a,b; Valeri et al, 1973).

Therapeutic Effectiveness

The index of therapeutic effectiveness refers to the percentage of originally collected donor red cells that have a potential for normal long-term survival in the recipient (Figure 2) (Valeri, 1970). To calculate the index the recovery *in vitro* is multiplied by the 24-hour posttransfusion survival of the recovered red cells. FIG. 2

RESULTS

The P_{50} value of the oxyhemoglobin dissociation curve of normal red cells collected in heparin and washed prior to testing was 27 $\pm$ 1 mm Hg (mean $\pm$ standard deviation), the carboxyhemoglobin level 1 to 2%, the red cell ATP level 3.9 $\pm$ 1.0 umoles per gram of hemoglobin, and the red cell 2,3 DPG level 12 $\pm$ 1.2 umoles per gram of hemoglobin.

All units of washed freeze-preserved red cells were sterile irrespective of the processing method.

Figures 3 and 4 outline the external dilution principles for washing the high and low glycerol red cells in the IBM Blood Processor, the Fenwal Elutramatic, and the Haemonetics Blood Processor 15. Also shown are the washing principle, the set-up time, the washing time, the volume of sodium chloride solutions, total time for preparation excluding the time of thawing, and the number of units that each machine could handle at one time. FIG. 3 FIG. 4

Figures 5A, 5B, 5C, 5D, 6A, 6B, 6C, 7A, 7B, and 7C show the various aspects of freeze-preservation of human red cells using 40% W/V glycerol and storage at -80 C, or 20% W/V glycerol and storage at -150 C. The red cells were stored at 4 C in either ACD or CPD for up to 5 days before freeze-preservation with the high or low glycerol method. After thawing and washing they were stored in a sodium chloride-glucose-phosphate solution for up to 4 days at 4 C. The recovery *in vitro*, 24-hour posttransfusion survival value, and FIG. 5A FIG. 5B FIG. 5C FIG. 5D FIG. 6A FIG. 6B FIG. 6C FIG. 7A FIG. 7B FIG. 7C

oxygen transport function were not significantly different in the red cells washed by the various systems. The red cell 2,3 DPG and ATP levels, blood pH, and red cell pH were similar in high and low glycerol red cells (Figures 5B, 6B, and 7B). The supernatant hemoglobin level was higher in low glycerol red cells than in high glycerol red cells both on the day of washing and for 24 hours after storage in the resuspension medium at 4 C (Figures 5C, 6C and 7C).

After freeze-preservation, washing, and storage in sodium chloride-glucose-phosphate for 4 days at 4 C, the non-rejuvenated red cells had 24-hour posttransfusion survivals of about 85%, a slight reduction in oxygen transport function, and an increase in spontaneous hemolysis *in vitro* (Figure 6A).

Figures 8A and 8B show the 2,3 DPG and ATP levels of red cells after storage in ACD or CPD at 4 C. After storage in CPD at 4 C for 3 to 5 days the red cell 2,3 DPG begins to fall, whereas red cells stored in ACD exhibit a fall in the 2,3 DPG level after only 1 or 2 days of storage at 4 C. The level of red cell 2,3 DPG after thawing and washing is similar to that at the time of glycerolization and freezing. After storage at 4 C for 3 to 5 days CPD red cells can be incubated in PIGPA (Solution A) for 1 hour at 37 C to increase their 2,3 DPG levels to 1-1/2 to 2 times normal, and their ATP levels to 125% the normal value. In fact, red cells can be stored at 4 C in ACD or CPD for as long as 28 days before rejuvenation with PIGPA (Solution A), glycerolization and freezing. After rejuvenation

FIG. 8A
FIG. 8B

these outdated red cells have 2,3 DPG levels of 75 to 80% of normal, and ATP levels 125% the normal value.

Figure 8C shows the 24-hour posttransfusion survival and lifespan of high and low glycerol red cells after freezing, washing, and storage in sodium chloride-glucose-phosphate at 4 C for 24 hours. Red cells that were stored in ACD or CPD at 4 C for 3 to 5 days before freeze-preservation, washing, and post-thaw storage for 24 hours had 24-hour posttransfusion survival values of about 90%, and lifespan values of about 100 days. When the CPD red cells that had been stored for 3 to 5 days were rejuvenated with PIGPA (Solution A) before freeze-preservation, etc., the 24-hour posttransfusion survival was about 85%, and the lifespan was about 100 days. When, on the other hand, red cells that had been stored in ACD or CPD for 28 days at 4 C were rejuvenated with PIGPA (Solution A) before freeze-preservation, etc., the 24-hour posttransfusion survival was 78 to 80%, and the lifespan was about 100 days. When these <u>indated-rejuvenated</u> and <u>outdated-rejuvenated</u> red cells were kept in the sodium chloride-glucose-phosphate solution for as long as 48 hours, the 24-hour posttransfusion survivals were at least 70% and the long-term survival was about 100 days (Figures 9A-9D). When they were kept at 4 C for as long as 4 days after washing, the 24-hour posttransfusion survivals were at least 70% (Figure 6A), but there was an increase in hemolysis <u>in vitro</u>. The red cells were concentrated by centrifugation prior to transfusion to remove all the visible supernatant solution that contains the products of hemolysis.

FIG. 9A
FIG. 9B
FIG. 9C
FIG. 9D

After storage at 4 C in ACD or CPD for up to 28 days, red blood cells can be biochemically modified with a rejuvenation solution (PIGPA, Solution B) which contains pyruvate, inosine, glucose, phosphate and adenine, to increase the red cell 2,3 DPG and ATP levels to 1-1/2 times normal (Figures 10A and 10B) and can be freeze-preserved with the high or low glycerol method. After thawing and washing the red cells can be stored at 4 C in a sodium chloride-glucose-phosphate solution for 24 hours. Red cells that were stored at 4 C for 14 to 16 days before freezing had posttransfusion survivals of about 90%. When the red cells were stored for 22 to 26 days before freezing the value was about 80% (Figure 10C).

FIG. 10A
FIG. 10B
FIG. 10C

All of the washing procedures reported here successfully remove the additives that are used in the rejuvenation process. Tables 3-7 and Figure 5D show the freeze-thaw and freeze-thaw-wash recovery of the red cells, the levels of residual supernatant hemoglobin, the levels of extracellular and red cell potassium, and of uric acid, inorganic phosphorus, lactate, hypoxanthine, inosine, and glycerol, and the residual ^{125}I radioactivity. With all three wash systems at least 90% of the red cells are recovered. The freeze-wash and freeze-thaw-wash recoveries, and the residual supernatant hemoglobin levels on the day of washing were higher in the low glycerol red cells than in the high glycerol red cells. The residual supernatant hemoglobin level was slightly higher in red cells washed in the Haemonetics Blood Processor 15 than in those

TABLES 3-

washed in the Fenwal Elutramatic or the IBM Blood Processor. There was a correlation between the red cell potassium ion levels and the length of storage at 4 C prior to freezing, and these levels were similar in high and low glycerol red cells (Figures 5B, 6B, and 7B). Red cells stored for 28 to 35 days before freeze-preservation had lower potassium ion levels than those stored for 3 to 5 days (Figures 5B, 6B, and 7B). Rejuvenation of the red cells did not improve the potassium ion level, but it did improve the 2,3 DPG and ATP levels. The potassium ion level was about 10% lower after washing than after thawing.

DISCUSSION

In our laboratory we have utilized three important principles in the freeze-preservation of human red cells with high or low concentrations of glycerol.

(1) Concentration of red cells to hematocrits of about 90 V% before glycerolization.

(2) Dilution of red cells with a sodium chloride solution prior to recovery, and washing with sodium chloride solutions in any one of three commercially available systems.

(3) Second dilution by on-line delivery of the once-diluted red cells and the wash solution in the Haemonetics or the Fenwal Elutramatic washing systems using the continuous-flow principle.

Red cell concentrates with hematocrits of 90 V% were prepared by removing all of the plasma, both to salvage the plasma and to purify the red cells. The low glycerol red cells with a final glycerol concentration of about 20% W/V are prepared from a solution of 35% W/V glycerol which is stored at 22 C or at 37 C and is added with agitation to red cell concentrates that have been stored at room temperature (22 to 25 C) for at least 2 hours (one-step addition). The high glycerol red cells with a final glycerol concentration of about 40% W/V are prepared from a solution of 57% W/V glycerol which is stored at 22 C or at 37 C and is added in two steps with agitation to red cell concentrates that have been stored at room temperature (22 to 25 C) for at least 2 hours (two-step addition). With the

two-step method, the initial volume of the 6.2 M glycerol solution is matched to the weight of the concentrated red cells using a nomogram, and the glycerol-red cell mixture equilibrated for at least 10 minutes before the balance of the solution can be added (Table 2). Contrary to the suggestion of Meryman and Hornblower (1972), we found that no special stylette was required to add the glycerol to the red cell concentrates. The goal in washing glycerolized red cells is to use the smallest volume of wash solution, and to recover the maximum number of red cells in the shortest period of time. The washing process also removes the products of hemolysis, reduces the glycerol concentration to less than 1% W/V, and reduces the residual protein, the white cells and platelets, and the anticoagulant and additives (Tables 3-7) (Valeri, 1970; Valeri and Zaroulis, 1972a,b; Valeri, 1974b). The dilution method depends upon the system used to wash the red cells. With the IBM Blood Processor and the Fenwal Elutramatic we used the two-step dilution, and with the Haemonetics Blood Processor 15 we used the one-step method (Valeri, 1973a,b; Valeri, in press). When the high glycerol red cells were diluted by the two-step method prior to washing in the disposable polycarbonate bowl in the Haemonetics Blood Processor 15, the amount of solution required for washing was the same as that used with the one-step method, but the two-step method was more time-consuming. For this reason, we do not recommend the two-step dilution when the Haemonetics Blood Processor 15 is used and, in addition, recommend that the Haemonetics Blood Processor not be automated. When a programmed

pump is added to the system to deliver the wash solutions, the otherwise simple method is made more complex. Because washing is not as efficient in the polycarbonate bowl, 1.0 liter more wash solution is needed with the Haemonetics Blood Processor 15 than is needed with the IBM Blood Processor or the Fenwal Elutramatic. The hypoxanthine level in rejuvenated red cells that were washed in the Haemonetics Blood Processor 15 was about twice that in red cells washed in the IBM Blood Processor or the Fenwal Elutramatic (Tables 5, 6, and 7).

We used a 5.6% sodium lactate solution to wash 20% W/V glycerolized red cells in previous studies (Runck and Valeri, 1972; Valeri, 1973a,b), but we are currently using a 3.2% sodium chloride solution which has an osmotic pressure equal to 5.6% sodium lactate (Valeri, 1973a,b). On-line dilution of the blood and the wash solution is a very important principle of the continuous-flow systems. We have found that washing is made much simpler when a Y-set is used with the Fenwal Elutramatic, and when a bypass harness and gravity flow washing are used with the Haemonetics Blood Processor 15.

When operated in the manner described, all three washing systems reduced the residual glycerol concentration to 0.5% W/V or less (Tables 3-7). In addition to removing the glycerol and the additives used for rejuvenation, washing also reduces the residual proteins, the Australia antigen if present, the residual supernatant hemoglobin, and extracellular potassium ion. No matter what washing system was used, the recovery <u>in vitro</u> of the freeze-preserved red cells was 90% or greater.

We concluded that red cells can be stored in CPD at 4 C for 3 days before freeze-preservation with high or low concentrations of glycerol, washed in any of the three mentioned systems, and then stored in a sodium chloride-glucose-phosphate solution at 4 C for as long as 4 days before transfusion, and have excellent posttransfusion survival values and normal or slightly decreased oxygen transport function. There is a rapid fall in the red cell 2,3 DPG level of ACD red cells after 2 days of storage at 4 C; this level does not fall in CPD red cells until after about 5 days of storage. The 2,3 DPG level does not change significantly from the time of freezing to the time of washing. Indated-rejuvenated red cells had 2,3 DPG levels that were 1-1/2 to 2 times normal and ATP levels that were 125% to 150% of the normal value depending upon whether PIGPA Solution A or B was used (Figures 8A, 8B, 10A and 10B). Outdated-rejuvenated red cells had 2,3 DPG levels that were 80 to 150% of normal, and ATP levels that were 125 to 150% of the normal value depending upon the composition of the rejuvenation solution (Figures 8A, 8B, 10A and 10B).

The oxygen transport function and posttransfusion survival of freeze-preserved red cells are influenced by numerous factors: the anticoagulant used for collection, the length of storage at 4 C prior to glycerolization, the rejuvenation procedure, the method of freezing, the length of storage in the frozen state, the method used to remove the glycerol, and the composition of the resuspension medium and the length of storage at 4 C after washing.

Liquid-stored blood that is approaching its restricted shelf-life can be rejuvenated with high concentrations of potentially toxic substances, and prepared for freeze-preparation (Figure 11) (Akerblom, 1967; Deuticke et al, 1971; Duhm et al, 1971; Valeri, 1973a,b, 1971, 1974a,b; Valeri and Zaroulis, 1972a,b). The potential toxicity of these rejuvenation substances does not pose a problem since the substances are removed during routine washing of the thawed red cells. The costs involved in rejuvenating outdated red cells, including the expense of hardware, software, and labor, are deemed justifiable, since this procedure makes possible the salvaging of universal donor group O Rh-positive and group O Rh-negative red cells that would otherwise be discarded. FIG. 11

Therapeutic Effectiveness of Red Cells with 1-1/2 to 2 Times Normal 2,3 DPG Levels and Decreased Affinity for Oxygen

Patients requiring therapeutic transfusion usually have red cells with elevated 2,3 DPG levels and decreased affinity for oxygen. Unless a patient has cardiopulmonary insufficiency, the level of red cell 2,3 DPG increases in proportion to the degree of red cell mass deficiency (Eaton et al, 1970; Gerlach et al, 1970; Hjelm, 1969; Valeri and Fortier, 1969). It has been shown that in assessing a red cell mass deficit, the peripheral red cell 2,3 DPG measurement is 2 to 3 times more sensitive than the peripheral venous hemoglobin and hematocrit measurements (Valeri and Fortier, 1969). Patients who have cardiopulmonary disease but no red blood cell deficits also have elevated red

cell 2,3 DPG levels (Oski et al, 1969; Valeri and Fortier, 1969). Patients with hypoxic or anemic hypoxia who have normal or elevated blood pH levels usually have peripheral red cells with decreased affinity for oxygen and 2,3 DPG levels that are increased to about twice normal.

Patients with anemic hypoxia who had red cells with 1-1/2 to 2 times normal 2,3 DPG levels showed no impairment of oxygen uptake by the red cells in the lungs, but there was an increase in oxygen release to tissue (Valeri and Collins, 1971). Preserved red cells with 2,3 DPG levels 1-1/2 to 2 times normal would provide the most beneficial treatment for these patients. When 6 units of red cells with 1-1/2 to 2 times normal 2,3 DPG levels were administered to correct red cell mass deficits, the 2,3 DPG level remained elevated for 3 days after the transfusion. The levels may remain elevated for even longer periods, depending upon the metabolic and cardiopulmonary condition of the patient.

Of 38 patients who received 2 to 6 units of red cells that had been rejuvenated with PIGPA (Solution A), none exhibited any significant increase in serum uric acid concentration for 3 days after transfusion (Table 8). We have been administering rejuvenated red cells to patients for over 4 years and have encountered no problems of contamination, pyrogenic reactions, or other untoward side effects. Approximately 250 patients have received about 1500 units of <u>indated-rejuvenated</u> or <u>outdated-rejuvenated</u> red cells freeze-preserved with high or low concentrations of glycerol, and we have found that these red cells increase the recipient's red cell mass and improve oxygen transport.

TABLE 8

Red Cells With 1-1/2 Times Normal 2,3 DPG Levels During Extracorporeal Circulation

Under a collaborative study between the Naval Blood Research Laboratory and the Thoracic Surgical Service at Boston University Medical Center, patients subjected to extracorporeal perfusion for surgical correction of coronary artery disease were given their own fresh red cells as well as washed freeze-preserved red cells with high (1-1/2 times normal) 2,3 DPG levels to determine the effect of high 2,3 DPG red cells on myocardial function (Dennis et al, in press). For comparative evaluation other patients were given their own fresh red cells and stored red cells with 70% of normal 2,3 DPG levels that were not washed before transfusion.

One or 2 units of blood were drawn from each patient prior to surgery, and this blood was stored in the CPD anticoagulant at room temperature for about 4 hours during extracorporeal bypass. During restoration of the patient's blood volume with crystalloid solution, the cardiac output and pulmonary artery wedge pressure were measured. During cardiopulmonary bypass and for the next 24 hours, each patient was given his own fresh blood and 6 units of washed freeze-preserved red cells with 1-1/2 times normal 2,3 DPG levels, or his own fresh blood and 6 units of unwashed red cell concentrates that had been stored in CPD for about 5 days and had 2,3 DPG levels that were about 70% of normal. The mean cardiac index and the mean pulmonary artery wedge pressure were similar in the two groups prior to surgery.

At the end of cardiopulmonary bypass some of the blood remaining in the pump was infused into the patient to restore his blood volume. When red cells with high 2,3 DPG levels were transfused during and immediately after extracorporeal bypass there was a significant increase in oxygen consumption and a slight insignificant increase in cardiac index at the end of bypass. The _in vivo_ P_{50} value was about 3 mm Hg higher in the patients who received high 2,3 DPG red cells than in those who received red cells with reduced 2,3 DPG levels. However, there were no significant differences in mixed venous PO_2, hematocrit, body temperature, or blood lactate between the two groups.

During the first hour after coming off the pump, all the blood that remained in the pump was transfused to maintain systemic arterial blood pressure and pulmonary artery wedge pressure. When the mean pulmonary artery wedge pressure was similar in the two groups the patients with high 2,3 DPG red cells showed a mean cardiac index that was about 35% higher than that in patients with low 2,3 DPG red cells after transfusion. Patients who had been treated with isoproterenol or who had received intra-aortic balloon assistance were eliminated from these analyses.

The blood volume remained relatively stable for one to 6 hours after bypass, and during this time the mean cardiac index and mean pulmonary artery wedge pressure were similar in the two groups. Twenty-four hours after extracorporeal bypass the

patients were infused with a colloidal plasma volume expander, and myocardial function was reassessed. At this time also the mean cardiac index and mean pulmonary artery wedge pressure were similar in the two groups.

The 2,3 DPG levels during the 4-hour post-perfusion period were about 12.5 uM/g Hb in the patients who received high 2,3 DPG red cells, and about 10.5 uM/g Hb in those who received red cells with reduced 2,3 DPG levels. Twenty-four hours post-perfusion the 2,3 DPG levels were about 14.5 uM/g Hb in the patients who received high 2,3 DPG red cells, and about 13.0 uM/g Hb in the patients who received red cells with reduced 2,3 DPG levels. The 2,3 DPG levels during the post-operative period are affected by: the recipient's acid-base, endocrine and cardio-respiratory state, degree of anemia, and phosphate metabolism. The better maintenance of 2,3 DPG in this study compared to that reported by others who used ACD blood during extracorporeal perfusion is due to the fact that CPD was used in our study (Bordiuk et al, 1971; Young et al, 1973). CPD has higher blood phosphorus and pH levels and thus higher 2,3 DPG levels.

Of the 11 patients who received red cells with reduced 2,3 DPG levels, 5 patients required isoproterenol, and 2 required intra-aortic balloon assistance. One patient died. Of the 11 patients who received high 2,3 DPG red cells, 2 patients required isoproterenol and 1 required intra-aortic balloon assistance. There were no deaths in this group.

After surgical correction of the blood flow to the myocardium and the transfusion of red cells with high 2,3 DPG red cells and

decreased affinity for oxygen, there was a dramatic improvement in the response of cardiac output to volume loading. Even a slight decrease in red cell affinity for oxygen appears to improve myocardial function following extracorporeal perfusion. After the transfusion of red cells with 1-1/2 times normal 2,3 DPG levels, the recipients showed a 15 per cent increase in red cell 2,3 DPG, a slight decrease in blood pH, and a significant decrease in red cell affinity for oxygen (an increase of 3mm in the *in vivo* p50 value). A 35% increase in cardiac output following volume loading of the heart accompanied these changes. However, myocardial function may be depressed after the transfusion of blood as a result of an acute decrease in ionizable calcium produced by the citrate in anticoagulated ACD or CPD blood (Cooper et al, 1973). CPD blood contains less citrate than ACD, and washing the red cells before transfusion removes this substance. Thus, the fact that the preserved red cells with high 2,3 DPG levels used in our study were washed before transfusion may have contributed to the improvement in myocardial function.

Hemoglobinemia may occur as a result of the supernatant hemoglobin infused with preserved red cells. This can be avoided by centrifuging the red cells to concentrate them to hematocrits of 90 V% and removing all the supernatant fluid before transfusion. The plasma hemoglobin levels were approximately 100 mg% in both groups. This finding was of particular significance to us in view of a previous study in our laboratory in which the transfusion of freeze-preserved red

cells during extracorporeal bypass surgery produced plasma hemoglobin levels of approximately 200 mg% (Valeri et al, 1970b). In that study, the red cells that were frozen and washed by the Huggins procedure had hematocrits of 70 V% at the time of transfusion. Hemoglobinuria was noted in all of the patients in that study, although renal function was not adversely affected. It is apparent from these studies that freeze-preserved red cells should be concentrated to hematocrits of 90 V%, and all the supernatant fluid removed before transfusion. When these red cells are administered through ultrapore filters with external pressure, red cell viability and function are satisfactory, and there is only minimal hemolysis.

The very important clinical observation of improved myocardial function in patients after the transfusion of 2,3 DPG-enriched red cells during extracorporeal bypass has prompted us to formulate more extensive studies to be carried out in the baboon. The red blood cells of baboons, like those of humans, can be biochemically modified so that they will have increased or decreased 2,3 DPG levels. Baboon red cells can be frozen and washed in much the same manner as human red blood cells. The baboons in our study will receive red cells with much lower 2,3 DPG levels than humans have received: patients have received red cells with 70% of normal 2,3 DPG levels, whereas the baboons in our study will receive red cells with 25% of normal 2,3 DPG levels. Comparisons will be made of these baboons and others who will receive red cells with 1-1/2 times normal 2,3 DPG. In addition to measurements of coronary blood flow, we will

measure PO_2, PCO_2, pH, blood lactate, and *in vivo* P_{50} in coronary sinus blood. These measurements have not been made in man because of the difficulty in obtaining coronary sinus blood.

In certain specific clinical situations, the oxygen transport of preserved red cells may be of particular importance. For example, when patients have restricted cerebral and cardiac responses that may not be able to compensate for the decreased oxygen delivery that usually occurs 4 hours after transfusion if the red cells have low 2,3 DPG levels; this may result in dangerously low venous PO_2 and tissue PO_2 levels. Red cells with normal or above normal 2,3 DPG levels should be administered to seriously ill patients; this will improve or at least maintain the venous PO_2 and tissue PO_2 levels, and yet not demand that the blood flow or the work of the heart be increased. Preserved red cells should be able to improve the oxygen transport immediately after transfusion and should not require compensation from the cardiorespiratory system or a reduction in venous oxygen tension.

In our laboratory, we stored washed freeze-preserved red cells in a sodium chloride-glucose-phosphate solution at 4 C for 24 hours, after which we concentrated the red cells by centrifugation and removed all of the visible supernatant. When these concentrated red cells were transfused through ultrapore filters, we observed satisfactory posttransfusion survival and oxygen transport function. In addition, there is an excellent rate of flow of the previously frozen washed red cells though the ultrapore filters (Valeri, to be published).

This simple approach makes it feasible to biochemically modify both indated and outdated red cells. The routine washing procedure removes the glycerol, at least 95% of the white cells and platelets, the isoagglutinins, the anticoagulant preservative and other additives, the products of hemolysis, and the protein and nonprotein plasma components (Valeri, 1970; Crowley and Valeri, 1974a,b,c).

SUMMARY

Preserved red cells are transfused to increase the delivery of oxygen to tissue. It is also essential that the preserved red cells circulate in order to increase the red cell mass and to improve the oxygen carrying capacity. The delivery of oxygen to tissue immediately after transfusion of preserved red cells depends to a great extent on their affinity for oxygen. Red cells stored in ACD for 7 days at 4 C maintain their ability to carry oxygen, but their ability to release it is impaired. It was not until 1967 that the correlation between oxygen transport function and the red cell 2,3 DPG level was appreciated. More recently, investigators have realized the importance of the oxygen delivering capacity of transfused red cells during the first 4 hours after transfusion. Red cells that have low 2,3 DPG levels and increased affinity for oxygen will increase the cardiac output and/or decrease the venous PO_2 for 4 hours after transfusion.

CPD-preserved red cells are more likely to have normal oxygen transport than are ACD-preserved red cells. During storage at 4 C the oxygen transport function of CPD red cells is maintained by purine nucleoside supplementation, and during freeze-preservation it is maintained with glycerol. By modifying the red cell biochemistry before freeze-preservation, it is possible to prepare viable red cells with 2,3 DPG levels that are 1-1/2 to 2 times normal and have decreased affinity for oxygen. Such red cells have acceptable posttransfusion survival and greater oxygen-releasing capacity for at least 72 hours after transfusion.

The well-being of certain patients may be placed in jeopardy of they are given preserved red cells that have increased affinity for oxygen, since the patient may not be able to meet the accompanying demand for increased blood flow, and the venous oxygen tension may fall to a critical level. Clearly, patients in hemorrhagic and septic shock, those subjected to extracorporeal circulation during cardiac surgery, and anemic patients with myocardial or cerebrovascular insufficiency can be handled most efficaciously by treating them with red cells that have 2,3 DPG levels that are either normal or about twice normal.

TABLE 1

Solutions Used to Biochemically Modify Red Blood Cells After Storage in ACD or CPD at 4 C For As Long As 4 Weeks

PIGPA-SOLUTION A

Pyruvate	50 mmoles/liter
Inosine	50 mmoles/liter
Glucose	100 mmoles/liter
Na_2HPO_4	50 mmoles/liter
NaCl	9 grams/liter
Adenine	5 mmoles/liter
mOsm/kg	650
pH	7.2

PIGPA-SOLUTION B

Pyruvate	100 mmoles/liter
Inosine	100 mmoles/liter
Glucose	100 mmoles/liter
Na_2HPO_4	200 mmoles/liter
NaCl	5 grams/liter
Adenine	5 mmoles/liter
mOsm/kg	650
pH	7.2

A 50 ml aliquot of the rejuvenation solution was added to a unit of whole blood or a unit of concentrated red cells and incubated at 37 C for 1 hour prior to glycerolization and freezing

TABLE 2

NOMOGRAM WAS USED TO MATCH THE WEIGHT OF CONCENTRATED RED CELLS WITH A HEMATOCRIT VALUE OF ABOUT 90 V% TO THE VOLUME OF THE 6.2 M GLYCEROL SOLUTION

Weight of Concentrated Red Cells With Hematocrit Value of About 90 V% (gm)	Initial Volume of Glycerol Solution (ml)	Total Volume of Glycerol Solution (ml)
80-120	40	150
121-160	50	200
161-200	60	250
201-240	75	300
241-280	85	350
281-320	100	400

TABLE 3

MEASUREMENTS OF RED CELLS STORED IN ACD OR CPD AT 4 C FOR 3 TO 5 DAYS BEFORE FREEZE-PRESERVATION WITH 40% W/V GLYCEROL AT -80 C, AND WASHING IN ONE OF 3 COMMERCIAL SYSTEMS

		IBM BLOOD PROCESSOR (2.2 liters)	FENWAL ELUTRAMATIC (2.7 liters)	HAEMONETICS BLOOD PROCESSOR 15 (3.2 liters)
Freeze-Thaw Recovery (%)	M	98.0	97.8	97.3
	SD	3.7	1.0	1.8
	N	38	114	26
Freeze-Thaw-Wash Recovery (%)	M	92.8	90.0	90.8
	SD	2.4	2.7	5.7
	N	38	114	25
Supernatant Hemoglobin (mg%)	M	49	90	129
	SD	18	50	55
	N	38	114	26
Extra K^+ (mEq/l)	M	0.6	0.7	0.8
	SD	0.5	0.3	0.4
	N	38	112	26
Red Cell K^+ (mEq/10^{12} RBC)	M	7.5	7.5	7.6
	SD	0.9	0.7	0.6
	N	15	17	14
Uric Acid (mg%)	M	1.7	1.5	1.6
	SD	0.8	0.3	0.5
	N	16	38	14
Inorganic Phosphorus (mg%)	M	25	22	17
	SD	8	8	6
	N	16	40	14
Lactate (umoles/ml)	M	2.9	2.7	4.3
	SD	0.9	1.2	1.0
	N	16	38	10
Hypoxanthine (umoles/ml)	M	0.01	0.01	0.01
	SD	--	--	--
	N	16	38	10
Inosine (umoles/ml)	M	0.01	0.01	0.01
	SD	--	--	--
	N	16	38	10
Glycerol (g%)	M	0.21	0.21	0.34
	SD	0.13	0.08	0.02
	N	27	37	48
^{125}I Albumin Remaining (%)	M	0.17	0.22	0.15
	SD	0.12	0.16	0.06
	N	8	27	7

TABLE 4

MEASUREMENTS OF RED CELLS STORED IN ACD OR CPD AT 4 C FOR 3 TO 5 DAYS BEFORE FREEZE-PRESERVATION WITH 20% W/V GLYCEROL AT -150 C, AND WASHING IN ONE OF 3 COMMERICAL SYSTEMS

		IBM BLOOD PROCESSOR (1.5 liters)	FENWAL ELUTRAMATIC (1.7 liters)	HAEMONETICS BLOOD PROCESSOR 15 (2.5 liters)
Freeze-Thaw Recovery (%)	M	97.1	96.5	96.5
	SD	2.2	0.7	3.2
	N	10	69	8
Freeze-Thaw-Wash Recovery (%)	M	93.1	90.0	92.9
	SD	1.8	2.0	1.9
	N	10	69	8
Supernatant Hemoglobin (mg%)	M	106	140	155
	SD	38	90	28
	N	10	63	8
Extra K^+ (mEq/l)	M	0.8	0.9	1.0
	SD	0.3	0.6	0.4
	N	10	69	8
Red Cell K^+ (mEq/10^{12} RBC)	M	6.9	7.1	7.2
	SD	0.4	0.5	0.5
	N	4	8	4
Uric Acid (mg%)	M	1.4	1.5	1.3
	SD	0.4	0.4	0.4
	N	6	14	9
Inorganic Phosphorus (mg%)	M	17	17	18
	SD	4	6	3
	N	6	11	9
Lactate (umoles/ml)	M	1.0	0.9	1.0
	SD	0.6	0.4	0.5
	N	4	10	8
Hypoxanthine (umoles/ml)	M	0.01	0.01	0.01
	SD	--	--	--
	N	6	4	4
Inosine (umoles/ml)	M	0.01	0.01	0.01
	SD	--	--	--
	N	6	11	4
Glycerol (g%)	M	0.34	0.22	0.49
	SD	0.13	0.11	0.23
	N	43	44	28
^{125}I Albumin Remaining (%)	M	0.25	0.12	0.29
	SD	0.09	0.06	0.07
	N	6	6	6

TABLE 5

MEASUREMENTS OF RED CELLS STORED IN CPD AT 4 C FOR 3 TO 5 DAYS BEFORE REJUVENATION WITH PIGPA (SOLUTION A), FREEZE-PRESERVATION WITH 40% W/V GLYCEROL AT -80 C, AND WASHING IN ONE OF 3 COMMERICAL SYSTEMS

		IBM BLOOD PROCESSOR (2.2 liters)	FENWAL ELUTRAMATIC (2.7 liters)	HAEMONETICS BLOOD PROCESSOR 15 (3.2 liters)
Freeze-Thaw Recovery (%)	M	97.9	97.6	97.8
	SD	0.8	1.0	0.8
	N	12	67	9
Freeze-Thaw-Wash Recovery (%)	M	93.0	90.2	90.4
	SD	1.5	3.1	3.3
	N	12	67	9
Supernatant Hemoglobin (mg%)	M	92	103	125
	SD	50	52	60
	N	12	67	9
Extra K^+ (mEq/l)	M	0.9	0.8	0.6
	SD	0.6	0.4	0.2
	N	12	64	8
Red Cell K^+ (mEq/10^{12} RBC)	M	7.0	7.5	7.3
	SD	0.2	0.7	0.6
	N	3	17	6
Uric Acid (mg%)	M	1.0	1.7	1.3
	SD	0.5	0.2	0.3
	N	5	13	5
Inorganic Phosphorus (mg%)	M	23	22	22
	SD	6	6	6
	N	4	13	4
Lactate (umoles/ml)	M	2.0	1.4	3.2
	SD	0.5	0.5	0.6
	N	5	17	7
Hypoxanthine (umoles/ml)	M	0.11	0.06	0.30
	SD	0.04	0.03	0.08
	N	4	15	5
Inosine (umoles/ml)	M	0.01	0.01	0.01
	SD	--	--	--
	N	5	13	5
Glycerol (g%)	M	0.21	0.21	0.34
	SD	0.13	0.08	0.02
	N	27	37	48
^{125}I Albumin Remaining (%)	M	0.17	0.22	0.15
	SD	0.12	0.16	0.06
	N	8	27	7

TABLE 6

MEASUREMENTS OF RED CELLS STORED IN ACD OR CPD AT 4 C FOR 28 TO 35 DAYS BEFORE REJUVENATION WITH PIGPA (SOLUTION A), FREEZE-PRESERVATION WITH 40% W/V GLYCEROL AT -80 C, AND WASHING IN ONE OF 3 COMMERICAL SYSTEMS

		IBM BLOOD PROCESSOR (2.2 liters)	FENWAL ELUTRAMATIC (2.7 liters)	HAEMONETICS BLOOD PROCESSOR 15 (3.2 liters)
Freeze-Thaw Recovery (%)	M	96.2	97.6	97.2
	SD	1.7	0.6	1.5
	N	27	62	10
Freeze-Thaw-Wash Recovery (%)	M	90.8	90.6	91.4
	SD	2.2	2.6	3.0
	N	27	62	10
Supernatant Hemoglobin (mg%)	M	77	87	122
	SD	54	34	34
	N	27	55	10
Extra K^+ (mEq/l)	M	1.0	0.8	0.8
	SD	0.5	0.8	0.3
	N	27	55	10
Red Cell K^+ (mEq/10^{12} RBC)	M	5.1	5.9	6.2
	SD	0.9	0.4	0.7
	N	8	12	4
Uric Acid (mg%)	M	2.1	2.2	1.6
	SD	0.8	0.6	2.5
	N	13	13	4
Inorganic Phosphorus (mg%)	M	20	21	20
	SD	2	6	3
	N	13	13	4
Lactate (umoles/ml)	M	3.8	2.0	3.7
	SD	0.4	0.7	0.4
	N	12	13	4
Hypoxanthine (umoles/ml)	M	0.18	0.09	0.31
	SD	0.07	0.04	0.03
	N	10	12	4
Inosine (umoles/ml)	M	0.01	0.01	0.01
	SD	--	--	--
	N	10	12	4
Glycerol (g%)	M	0.21	0.21	0.34
	SD	0.13	0.08	0.02
	N	27	37	48
^{125}I Albumin Remaining (%)	M	0.17	0.22	0.15
	SD	0.12	0.16	0.06
	N	8	27	7

TABLE 7

MEASUREMENTS OF RED CELLS STORED IN ACD OR CPD AT 4 C FOR 28 TO 35 DAYS BEFORE REJUVENATION WITH PIGPA (SOLUTION A), FREEZE-PRESERVATION WITH 20% W/V GLYCEROL AT -150 C, AND WASHING IN ONE OF 3 COMMERICAL SYSTEMS

		IBM BLOOD PROCESSOR (1.5 liters)	FENWAL ELUTRAMATIC (1.7 liters)	HAEMONETICS BLOOD PROCESSOR 15 (2.5 liters)
Freeze-Thaw Recovery (%)	M	96.3	96.8	96.4
	SD	3.8	0.7	0.7
	N	21	34	4
Freeze-Thaw-Wash Recovery (%)	M	91.1	88.7	92.4
	SD	2.0	3.7	1.3
	N	21	34	4
Supernatant Hemoglobin (mg%)	M	103	114	108
	SD	36	43	15
	N	21	34	4
Extra K^+ (mEq/l)	M	0.8	0.7	0.9
	SD	0.3	0.2	0.1
	N	21	34	4
Red Cell K^+ (mEq/10^{12} RBC)	M	5.8	6.0	6.5
	SD	1.1	0.8	0.2
	N	3	12	4
Uric Acid (mg%)	M	2.0	1.8	1.2
	SD	0.5	0.3	0.2
	N	8	9	4
Inorganic Phosphorus (mg%)	M	19	26	19
	SD	1	4	1
	N	8	4	4
Lactate (umoles/ml)	M	1.2	1.1	1.2
	SD	0.5	0.2	0.6
	N	8	9	4
Hypoxanthine (umoles/ml)	M	0.32	0.20	0.52
	SD	0.12	0.05	0.09
	N	7	5	4
Inosine (umoles/ml)	M	0.01	0.01	0.01
	SD	--	--	--
	N	7	5	4
Glycerol (g%)	M	0.34	0.22	0.49
	SD	0.13	0.11	0.23
	N	43	44	28
^{125}I Albumin Remaining (%)	M	0.25	0.12	0.29
	SD	0.09	0.06	0.07
	N	6	6	6

TABLE 8

URIC ACID LEVELS BEFORE AND AFTER TRANSFUSION OF 2 TO 6 UNITS OF RED CELLS REJUVENATED WITH PIGPA SOLUTION AFTER STORAGE AT 4 C FOR 4 WEEKS

	Number of Units Transfused	Pre-transfusion	Immediately After transfusion	15 minutes	30 minutes	4 hours	24 hours	48 hours	72 hours
Mean	3.0	5.5	5.3	6.0	5.0	5.4	5.1	5.3	5.5
S.D.	1.0	2.0	2.0	2.2	1.4	2.3	1.5	1.1	1.8
S.E.	0.2	0.3	0.3	0.5	0.3	0.5	0.2	0.2	0.3
n	38	38	38	18	18	20	38	24	38

FIGURE 1

Combinations of procedures used in the preservation of human red cells. Factors that influence the quality of the red blood cells are: pre-freeze storage at 4 C, composition of the rejuvenation solutions, freezing methods, washing methods, and the length of postthaw storage at 4 C in sodium chloride-glucose-phosphate.

VALERI - FIGURE 1

PRE-FREEZE STORAGE AT +4C	REJUVENATION SOLUTION	FREEZING PROCEDURE	WASHING	POST-THAW STORAGE AT +4C IN NaCl-GLUCOSE-PHOSPHATE
ACD vs. CPD Hct: 40% vs. 70% vs. 90% Length of Storage: 2-4 days 28-30 days	PIGP, PIGPA: Solution A PIGPA: Solution B	40% W/V Glycerol and −80C Storage 20% W/V Glycerol and −150C Storage	Continuous-Flow Centrifugation a) Elutramatic b) ADL 10 & 15 Serial Centrifugation IBM	24 Hours 3 Days 7 Days

FIGURE 2

The index of therapeutic effectiveness (ITE) represents the number of collected red cells in the recipient's circulation 24 hours after transfusion.

VALERI - FIGURE 2

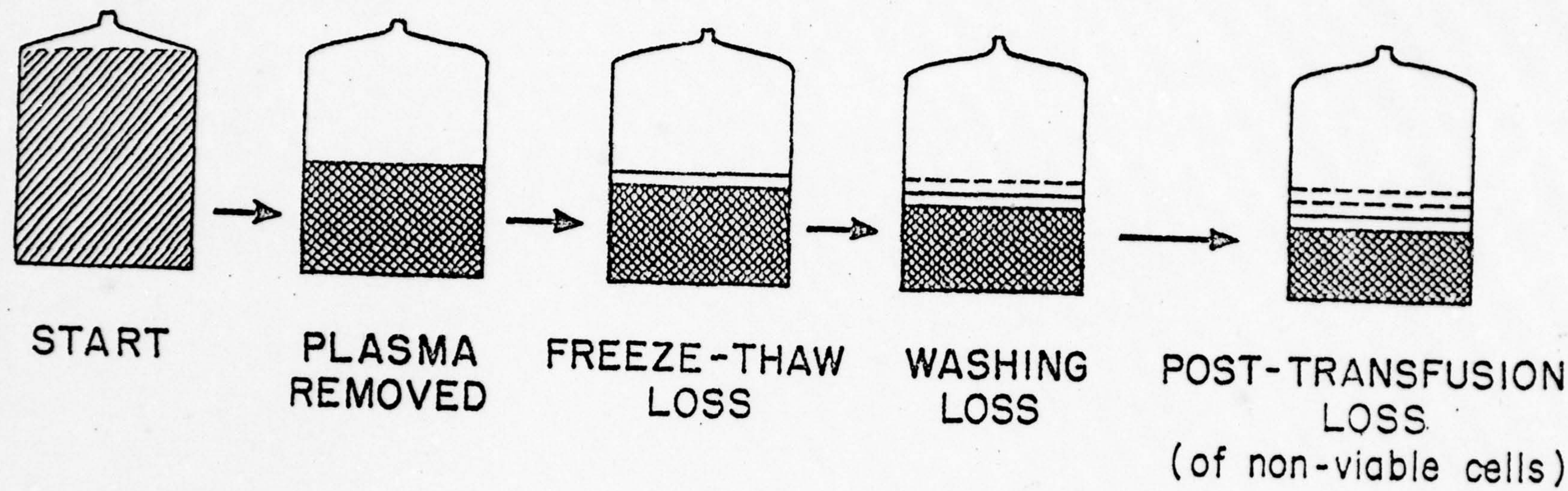
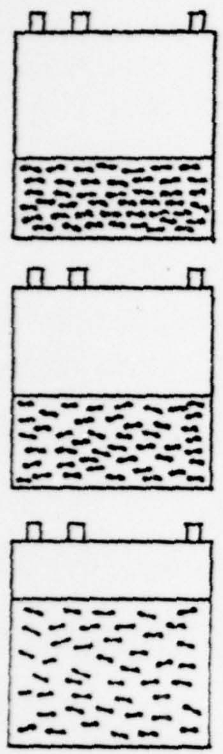


FIGURE 3

Dilution of 40% W/V glycerolized red cells first with 150 ml of 12 g% sodium chloride solution, and then with 450-500 ml of 1.6 g% sodium chloride solution in the Fenwal Elutramatic or in the IBM Blood Processor, prior to recovery and washing with sodium chloride solutions in these systems. Units washed in the Haemonetics Blood Processor 15 were diluted only once.

VALERI - FIGURE 3



RED CELLS CONTAINING
40% W/V GLYCEROL

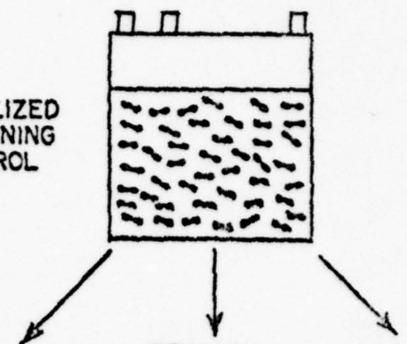
1st Dilution

ADDITION OF 150 ml OF 12% NaCl WITH AGITATION & EQUILIBRATED FOR AT LEAST 2 MINUTES AT ROOM TEMPERATURE

2nd Dilution

ADDITION OF 450-500 ml OF 1.6% NaCl WITH AGITATION

DILUTED GLYCEROLIZED RED CELLS CONTAINING ~18% W/V GLYCEROL

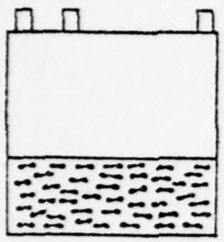


Washing Machine :	IBM	FENWAL ELUTRAMATIC	HAEMONETICS MODEL 15
Type of Washer :	AUTOMATED SERIAL CENTRIFUGATION	AUTOMATED CONTINUOUS FLOW CENTRIFUGATION	NON-AUTOMATED GRAVITY CONTINUOUS FLOW CENTRIFUGATION
No. of Units Washed In Each Machine :	1	2	1
Set Up Time (Min) :	~3-5	~8-10	~3
Washing Time (Min) :	~20	~25	~20
Total Time (Min) :	~25	~35	~25
Volume of NaCl Solutions (Liters) :	2.2	2.7	3.2

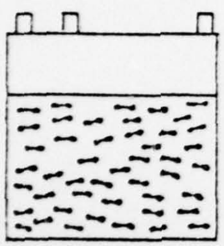
FIGURE 4

Dilution of 20% W/V glycerolized red cells with 250-500 ml of 3.2 g% sodium chloride solution prior to recovery and washing with sodium chloride solutions in one of three commercial systems.

VALERI - FIGURE 4

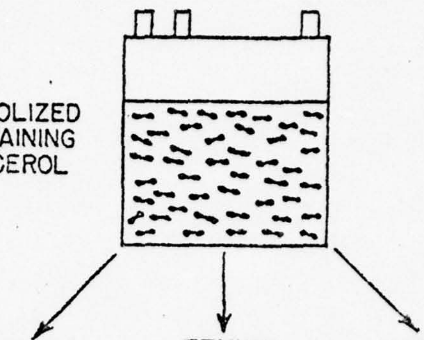


RED CELLS CONTAINING 20% W/V GLYCEROL



ADDITION OF 250-500 ml OF 3.2% NaCl WITH AGITATION

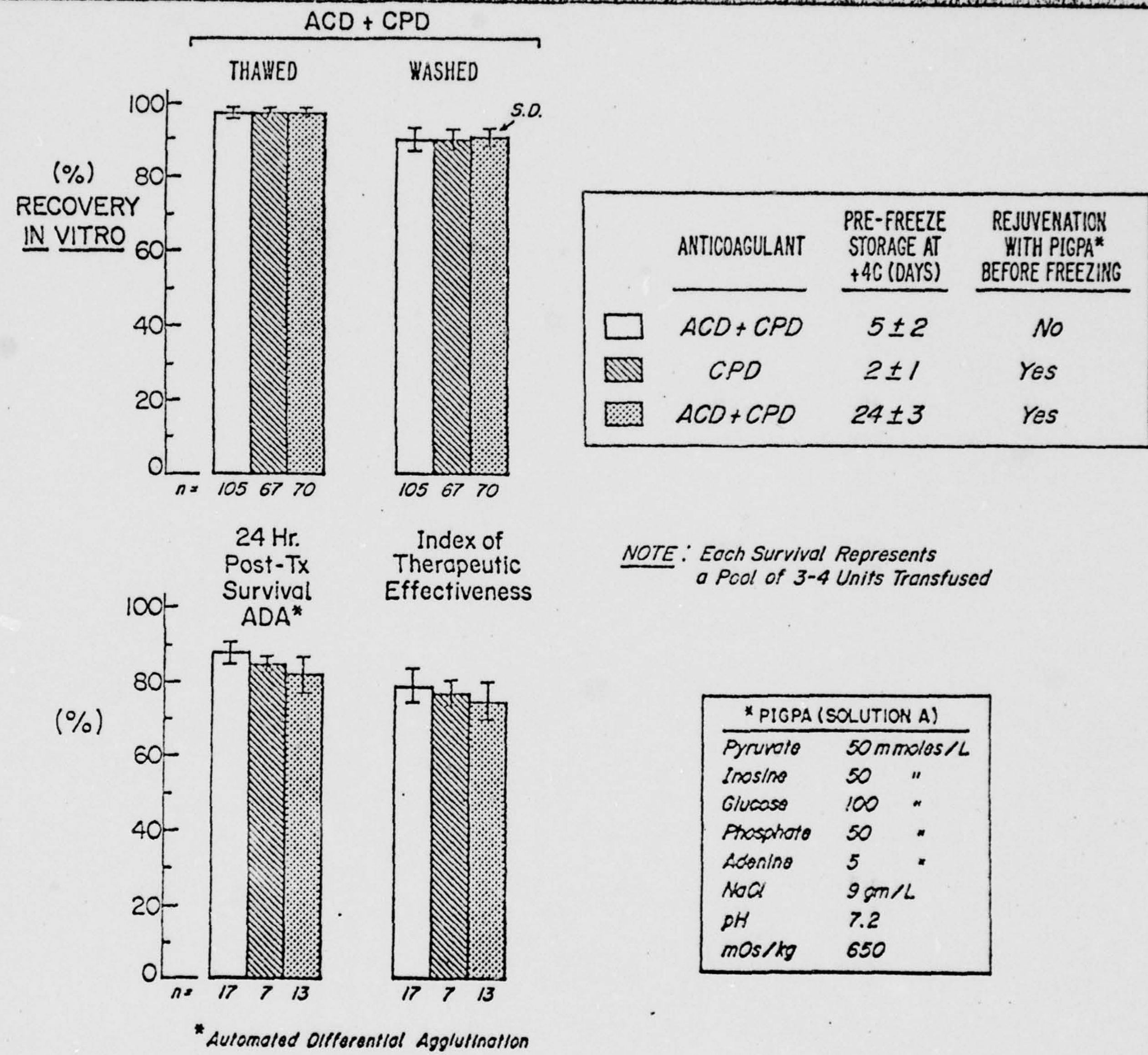
DILUTED GLYCEROLIZED RED CELLS CONTAINING ~10-14% W/V GLYCEROL



Washing Machine:	IBM	FENWAL ELUTRAMATIC	HAEMONETICS MODEL 15
Type of Washer:	AUTOMATED SERIAL CENTRIFUGATION	AUTOMATED CONTINUOUS FLOW CENTRIFUGATION	NON-AUTOMATED GRAVITY CONTINUOUS FLOW CENTRIFUGATION
No. of Units Washed In Each Machine:	1	2	1
Set Up Time (Min):	~3-5	~8-10	~3
Washing Time (Min):	~16	~16	~15
Total Time (Min):	~20	~25	~20
Volume of NaCl Solution (Liters):	1.5	1.7	2.5

FIGURE 5A

The freeze-thaw and freeze-thaw-wash recovery values, 24-hour posttransfusion survival value measured by automated differential agglutination, and index of therapeutic effectiveness following the transfusion of 3 to 4 units of preserved red cells. Red cells were stored in ACD or CPD for up to 28 days. Some units were rejuvenated with PIGPA (Solution A) before freeze-preservation with 40% W/V glycerol, and others were not. The frozen units were stored at -80 C for up to 9 months, and were washed in the Fenwal Elutramatic with 2.7 liters of sodium chloride solutions. The washed units were stored in a sodium chloride-glucose-phosphate solution at 4 C for 24 hours prior to transfusion.

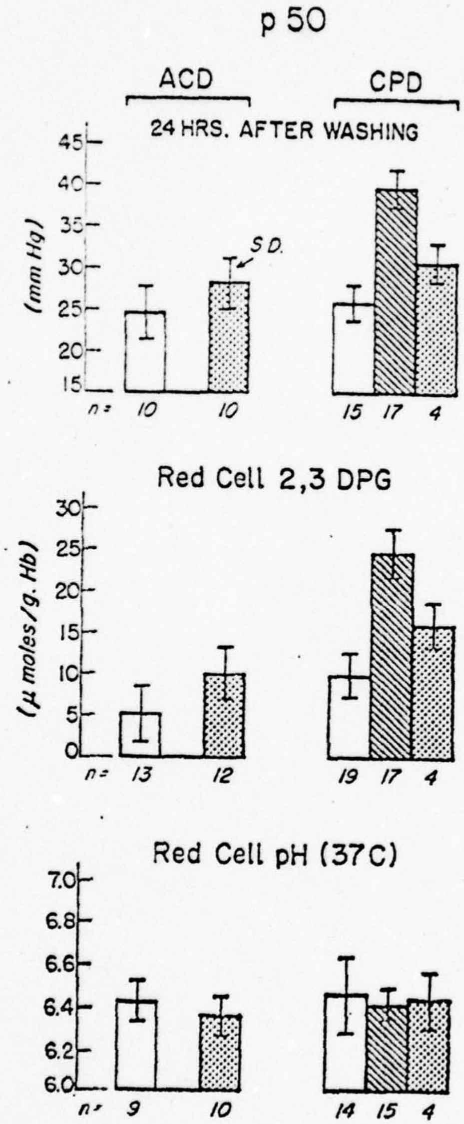


VALERI - FIGURE 5A

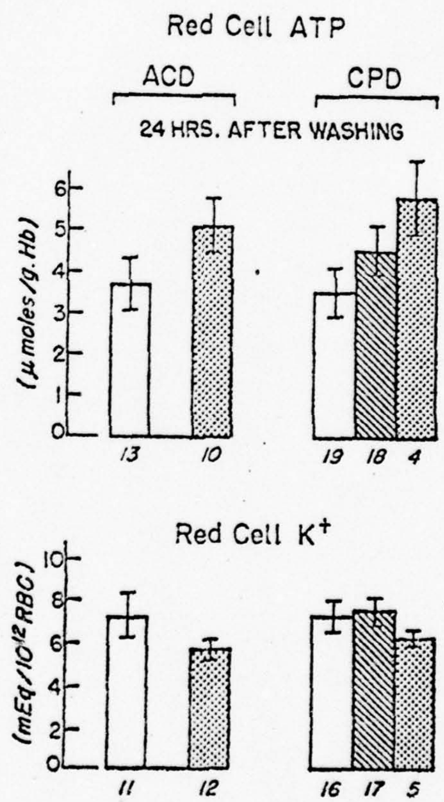
FIGURE 5B

P_{50}, red cell 2,3 DPG, ATP, pH, and potassium levels on the day of washing, and after storage at 4 C in sodium chloride-glucose-phosphate for 24 hours. Red cells were stored at 4 C in ACD or CPD for up to 28 days. Some of the units were rejuvenated prior to freezing as described in Figure 5A, and others were not.

VALERI - FIGURE 5B



	ANTICOAGULANT	PRE-FREEZE STORAGE AT +4C (DAYS)	REJUVENATION WITH PIGPA* BEFORE FREEZING
□	ACD + CPD	5 ± 2	No
▨	CPD	2 ± 1	Yes
░	ACD + CPD	24 ± 3	Yes

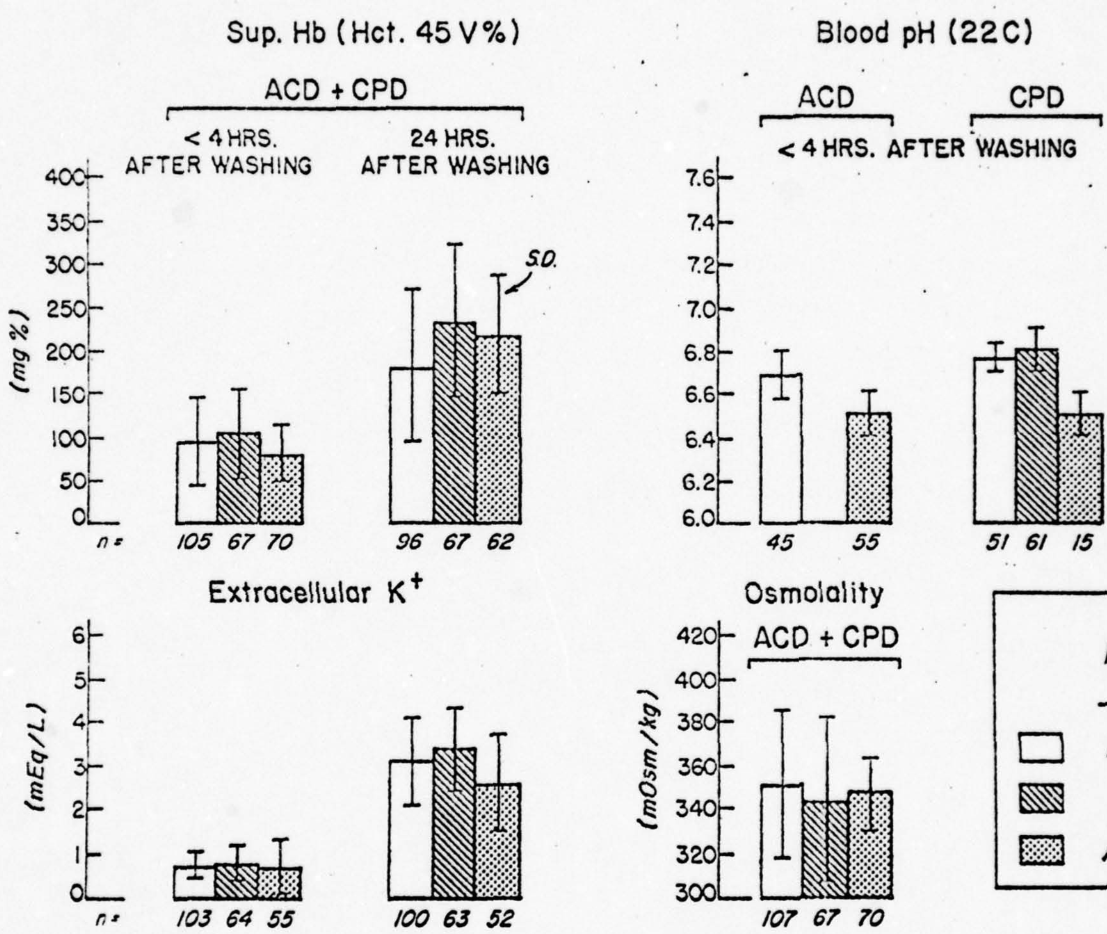


* PIGPA (SOLUTION A)	
Pyruvate	50 m moles/L
Inosine	50 "
Glucose	100 "
Phosphate	50 "
Adenine	5 "
NaCl	9 gm/L
pH	7 2
mOs/kg	650

FIGURE 5C

Supernatant hemoglobin, extracellular potassium, blood pH, and supernatant osmolality on the day of washing, and after storage at 4 C in sodium chloride-glucose-phosphate for 24 hours. Red cells were stored at 4 C in ACD or CPD for up to 28 days. Some of the units were rejuvenated prior to freezing as described in Figure 5A, and others were not.

VALERI - FIGURE 5C



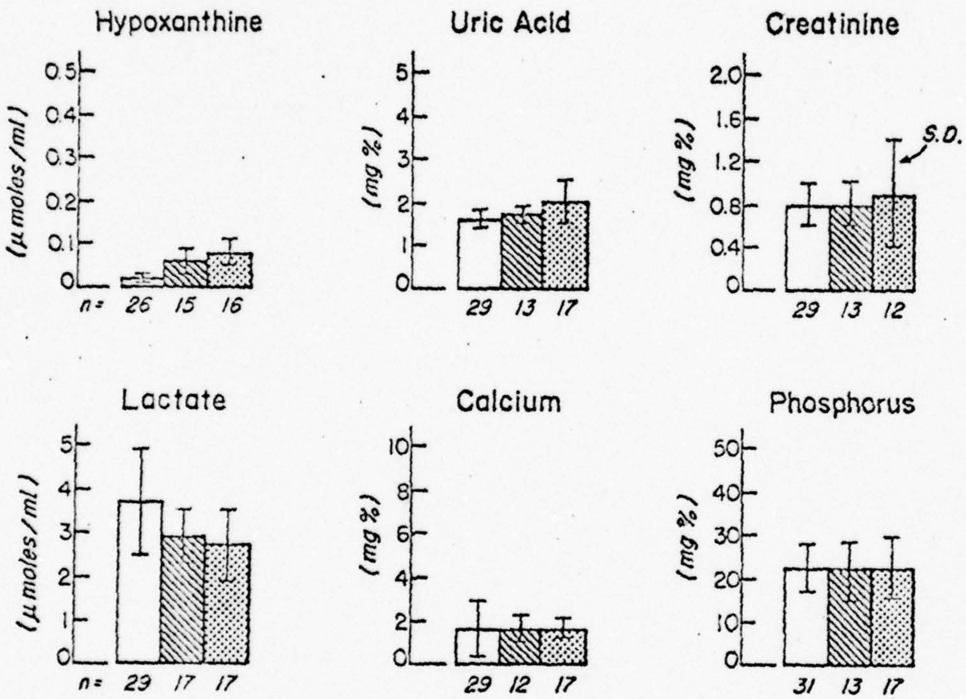
* PIGPA (SOLUTION A)	
Pyruvate	50 mmoles/L
Inosine	50 "
Glucose	100 "
Phosphate	50 "
Adenine	5 "
NaCl	9 gm/L
pH	7.2
mOs/kg	650

ANTICOAGULANT	PRE-FREEZE STORAGE AT +4C (DAYS)	REJUVENATION WITH PIGPA* BEFORE FREEZING
ACD + CPD	5 ± 2	No
CPD	2 ± 1	Yes
ACD + CPD	24 ± 3	Yes

FIGURE 5D

Hypoxanthine, uric acid, creatinine, lactate, calcium, and phosphorus levels on the day of washing, and after storage at 4 C in sodium chloride-glucose-phosphate for 24 hours. Red cells were stored at 4 C in ACD or CPD for up to 28 days. Some of the units were rejuvenated prior to freezing as described in Figure 5A, and others were not.

ACD & CPD 24 HOURS AFTER WASHING



* PIGPA (SOLUTION A)	
Pyruvate	50 m moles/L
Inosine	50 "
Glucose	100 "
Phosphate	50 "
Adenine	5 "
NaCl	9 gm/L
pH	7.2
mOs/kg	650

	ANTICOAGULANT	PRE-FREEZE STORAGE AT +4C (DAYS)	REJUVENATION WITH PIGPA* BEFORE FREEZING
	ACD + CPD	5 ± 2	No
	CPD	2 ± 1	Yes
	ACD + CPD	24 ± 3	Yes

FIGURE 6A

Recovery *in vitro* after thawing and after washing, 24-hour ^{51}Cr posttransfusion survival, and index of therapeutic effectiveness. Red cells were stored at 4 C in CPD for 3 to 28 days. Some of the units were rejuvenated with PIGPA (Solution A) before freeze-preservation with 40% W/V glycerol in an ionic medium, and others were not. The frozen units were stored at -80 C for about one month, and were washed in the Fenwal Elutramatic with 2.7 liters of sodium chloride solutions. The washed units were stored with a hematocrit value of about 40 V% at 4 C in sodium chloride-glucose-phosphate for up to 96 hours. On the day of autologous transfusion the red blood cells were concentrated by centrifugation and the hematocrit was adjusted to 90 V% by removal of all the visible supernatant solution.

VALERI - FIGURE 6A

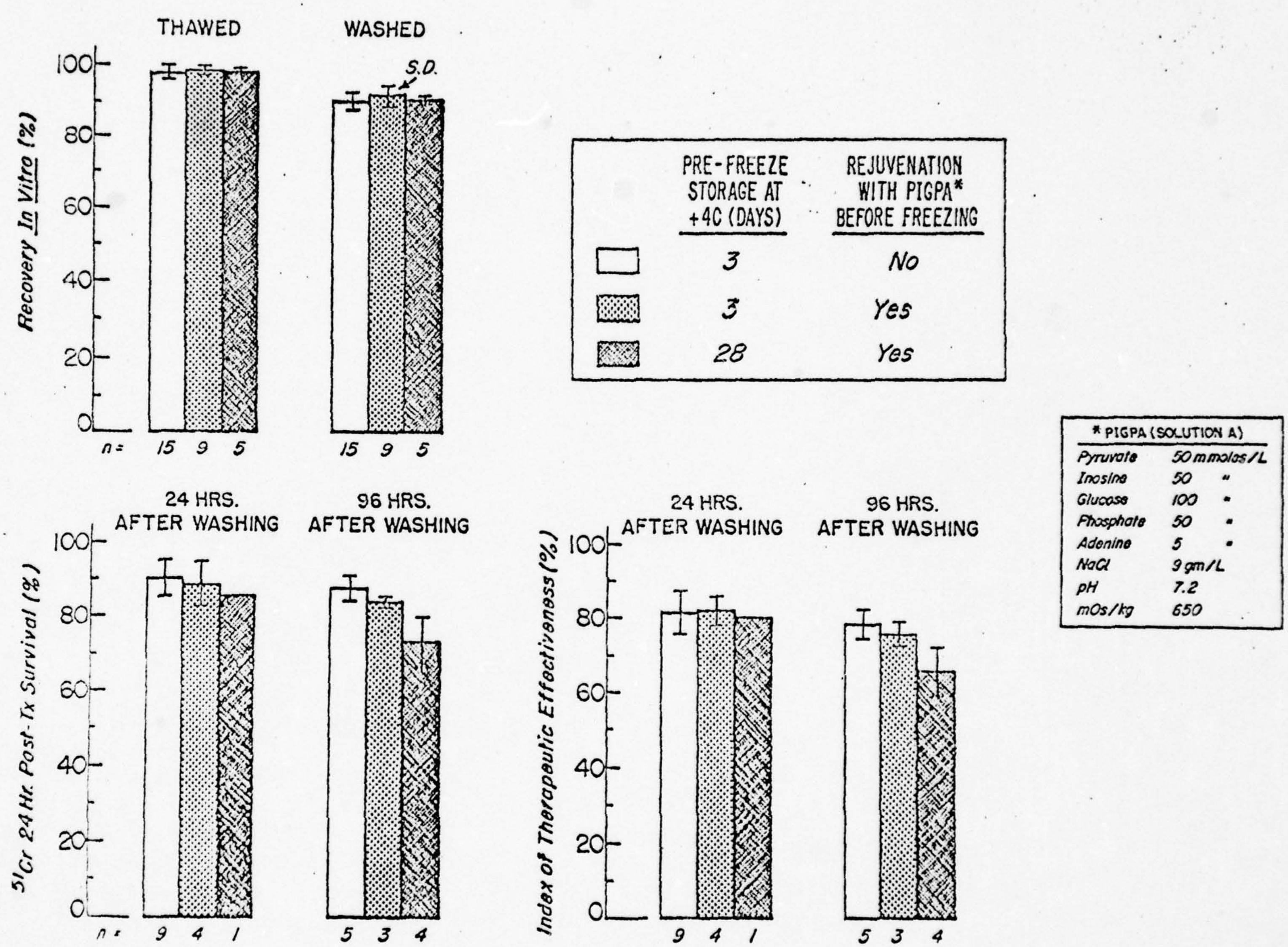


FIGURE 6B

P_{50} and red cell 2,3 DPG, ATP, pH, and potassium levels on the day of washing and after 96 hours of post-wash storage at 4 C. Red cells were stored at 4 C in CPD for 3 to 28 days. Some of the units were rejuvenated with PIGPA (Solution A) before freeze-preservation with 40% W/V glycerol in an ionic medium, and others were not. The frozen units were stored at -80 C for about one month, and were washed in the Fenwal Elutramatic with 2.7 liters of sodium chloride solutions. The washed units were stored at 4 C in sodium chloride-glucose-phosphate for up to 96 hours. On the day of autologous transfusion the red blood cells were concentrated by centrifugation and the hematocrit was adjusted to 90 V% by removal of all the visible supernatant solution.

VALERI - FIGURE 6B

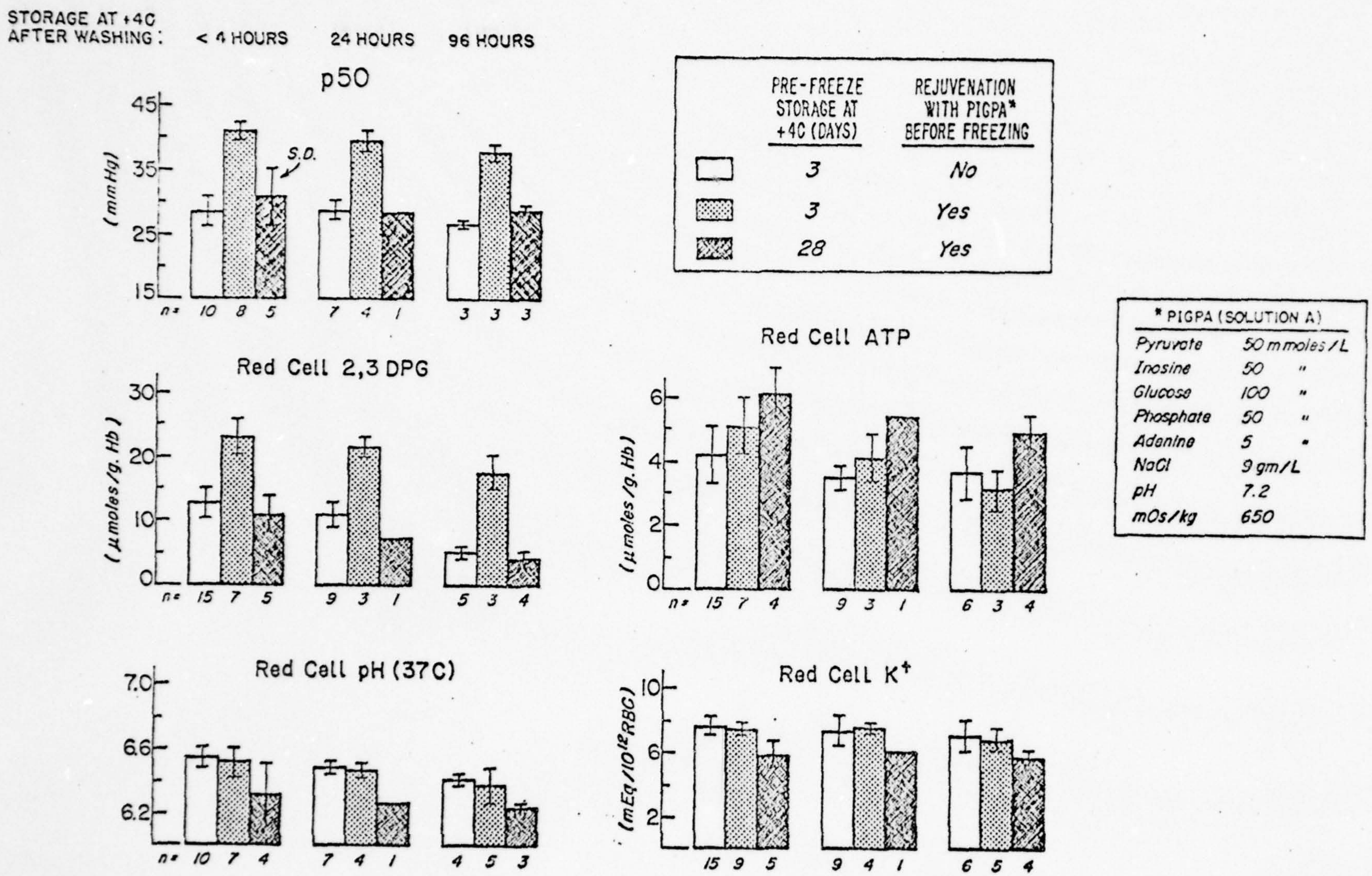


FIGURE 6C

Supernatant hemoglobin and extracellular potassium levels on the day of washing, and after 96 hours of post-wash storage at 4 C. Red cells were stored at 4 C in CPD for 3 to 28 days. Some of the units were rejuvenated with PIGPA (Solution A) before freeze-preservation with 40% W/V glycerol in an ionic medium, and others were not. The frozen units were stored at -80 C for about one month, and were washed in the Fenwal Elutramatic with 2.7 liters of sodium chloride solutions. The washed units were stored with a hematocrit value of 40 V% at 4 C in sodium chloride-glucose-phosphate at 4 C for up to 96 hours. On the day of autologous transfusion the red blood cells were concentrated by centrifugation and the hematocrit was adjusted to 90 V% by removal of all the visible supernatant solution.

VALERI - FIGURE 6C

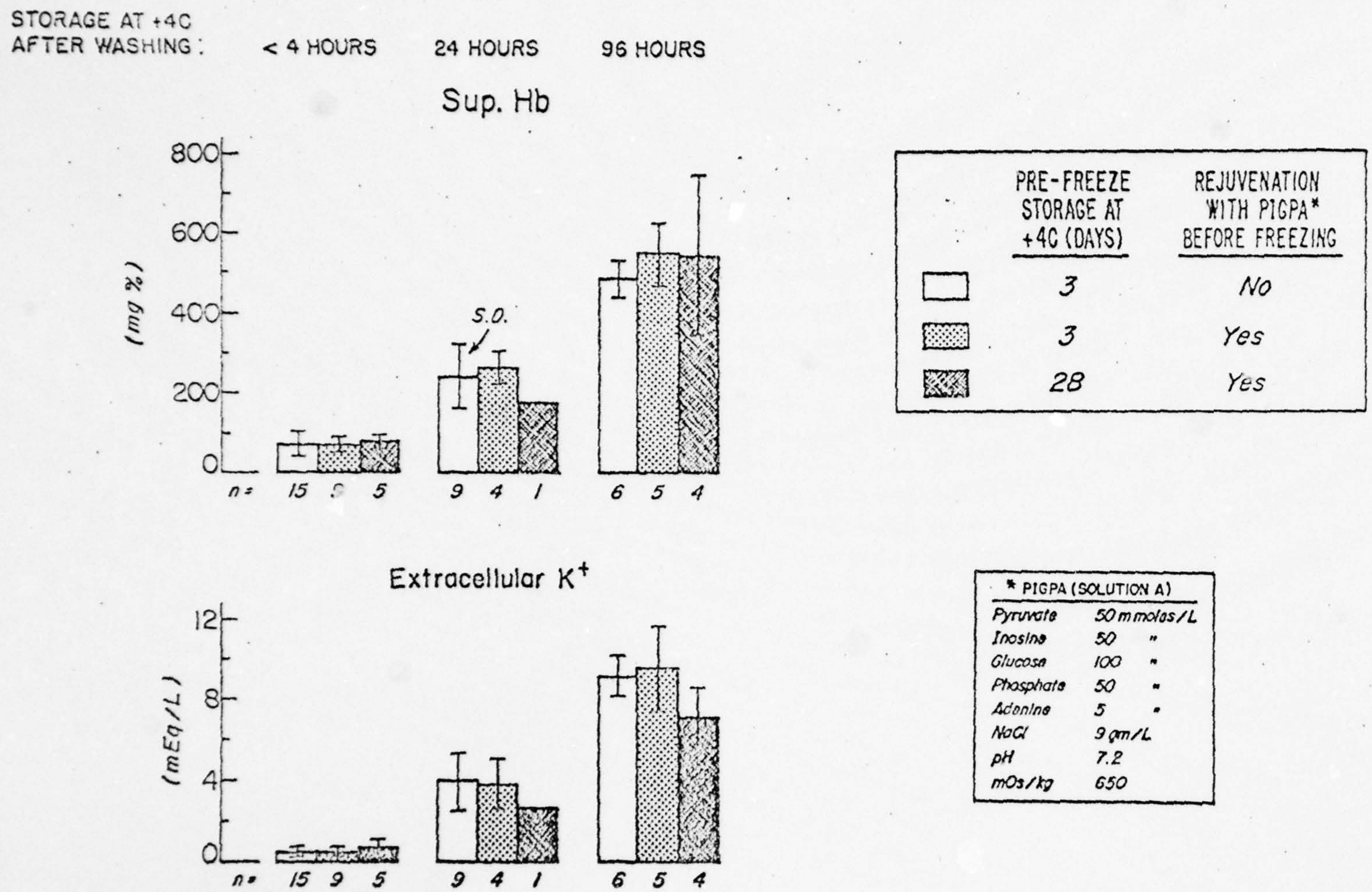
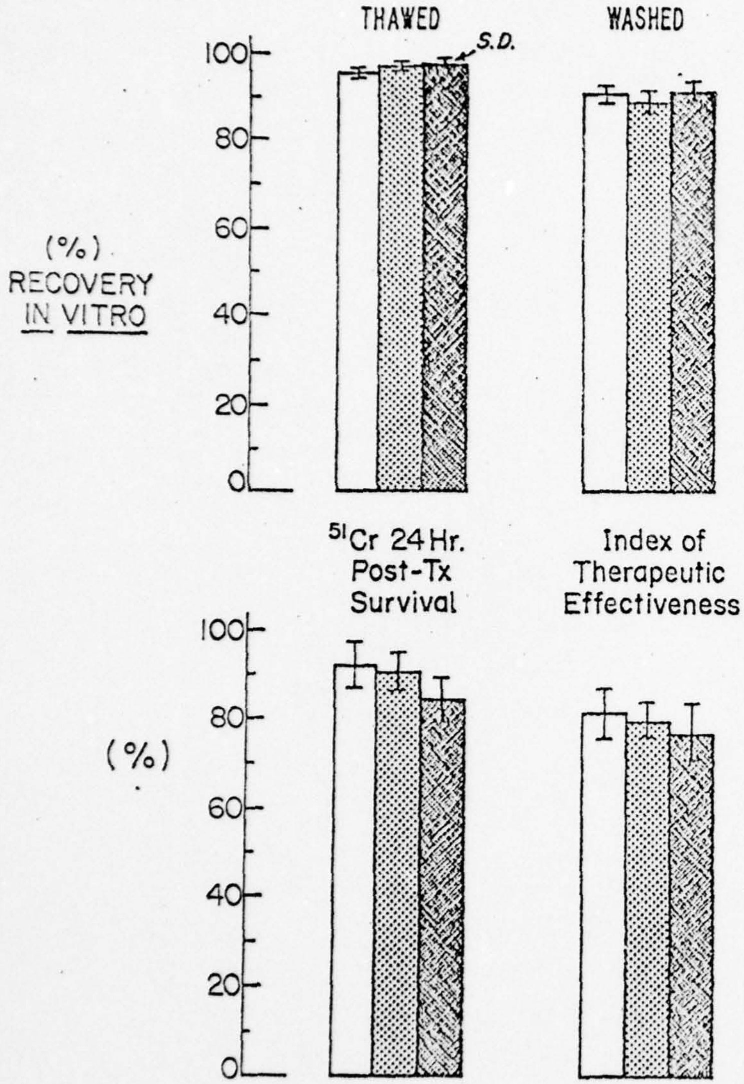


FIGURE 7A

Recovery *in vitro* after thawing and after washing, 24-hour ^{51}Cr posttransfusion survival, and index of therapeutic effectiveness. Red cells were stored in CPD at 4 C for 3 to 24 days. Some of the units were rejuvenated with PIGPA (Solution A) before freeze-preservation with 20% W/V glycerol, and others were not. The frozen units were stored at -150 C for about one month, and were washed in the Fenwal Elutramatic with 1.7 liters of sodium chloride solutions. The washed units were stored with a hematocrit value of about 40 V% at 4 C in sodium chloride-glucose-phosphate for 24 hours. On the day of autologous transfusion the red blood cells were concentrated by centrifugation and the hematocrit was adjusted to 90 V% by removal of all the visible supernatant solution.

VALERI - FIGURE 7A



	PRE-FREEZE STORAGE AT +4C (DAYS)	REJUVENATION WITH PIGPA* BEFORE FREEZING	N
	3	No	4
	3	Yes	4
	24	Yes	6

* PIGPA (SOLUTION A)	
Pyruvate	50 mmoles/L
Inosine	50 "
Glucose	100 "
Phosphate	50 "
Adenine	5 "
NaCl	9 gm/L
pH	7.2
mOs/kg	650

FIGURE 7B

P_{50} and red cell 2,3 DPG, ATP, pH, and potassium levels after storage at 4 C for 24 hours. Red cells were stored in CPD at 4 C for 3 to 24 days. Some of the units were rejuvenated with PIGPA (Solution A) before freeze-preservation with 20% W/V glycerol, and others were not. The frozen units were stored at -150 C for about one month, and were washed in the Fenwal Elutramatic with 1.7 liters of sodium chloride solutions. The washed units were stored with a hematocrit value of 40 V% at 4 C in sodium chloride-glucose-phosphate for 24 hours. On the day of autologous transfusion the red blood cells were concentrated by centrifugation and the hematocrit was adjusted to 90 V% by removal of all the visible supernatant solution.

VALERI - FIGURE 7B

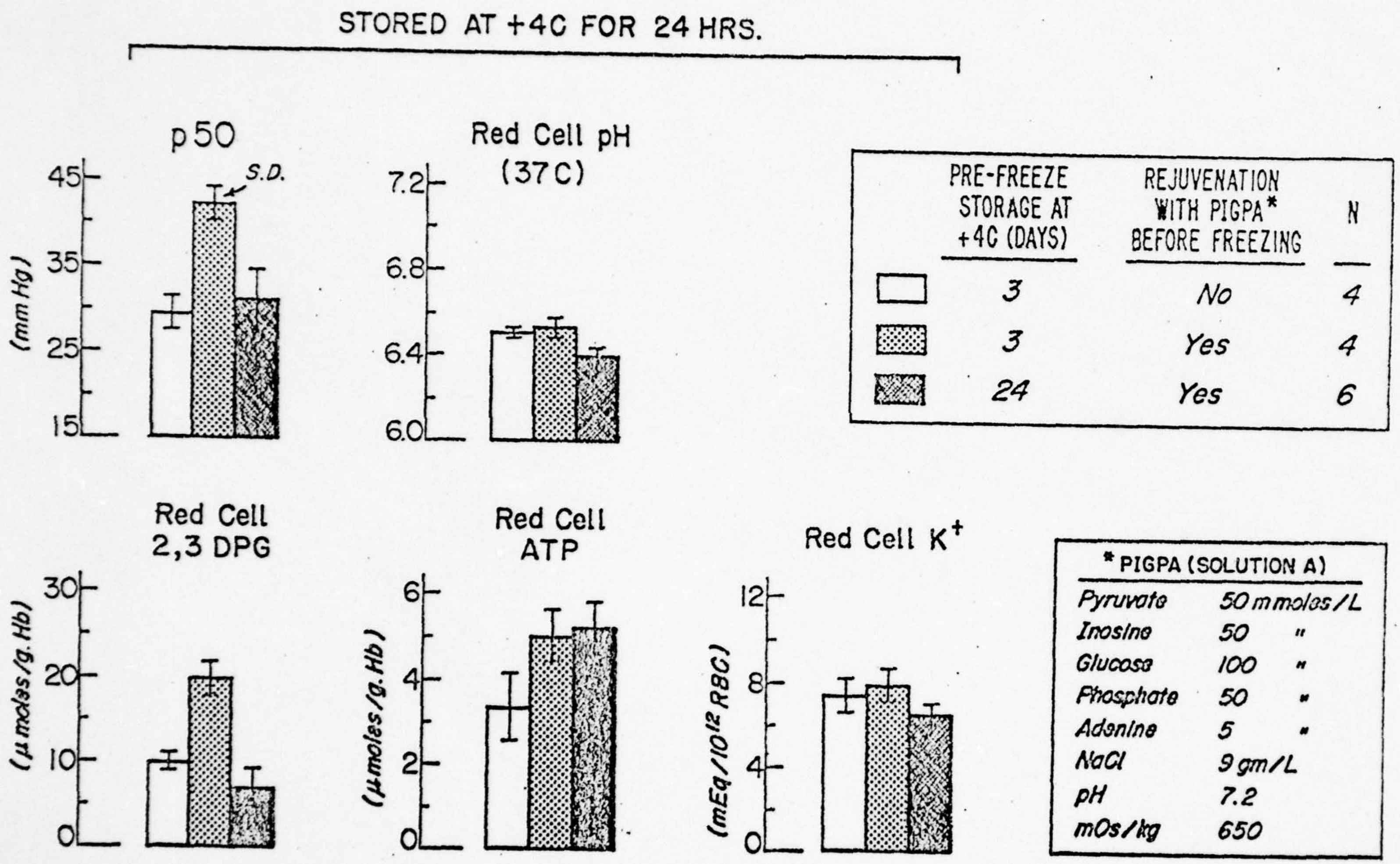
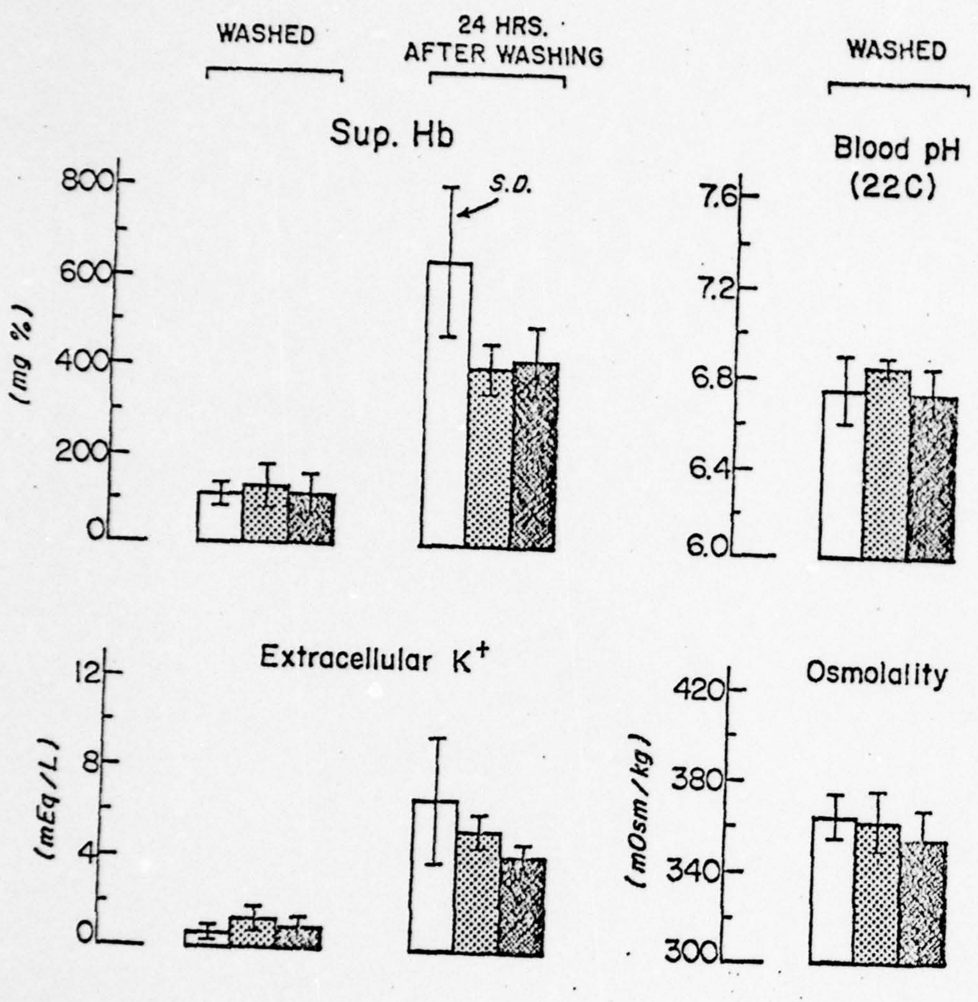


FIGURE 7C

Supernatant hemoglobin, extracellular potassium, blood pH, and supernatant osmolality on the day of washing, and after post-wash storage at 4 C for 24 hours. Red cells were stored in CPD at 4 C for 3 to 24 days. Some of the units were rejuvenated with PIGPA (Solution A) before freeze-preservation with 20% W/V glycerol, and others were not. The frozen units were stored at -150 C for about one month and were washed in the Fenwal Elutramatic with 1.7 liters of sodium chloride solutions. The washed units were stored with a hematocrit value of 40 V% at 4 C in sodium chloride-glucose-phosphate for 24 hours. On the day of autologous transfusion the red blood cells were concentrated by centrifugation and the hematocrit was adjusted to 90 V% by removal of all the visible supernatant solution.

VALERI - FIGURE 7C



	PRE-FREEZE STORAGE AT +4C (DAYS)	REJUVENATION WITH PIGPA* BEFORE FREEZING	N
(open)	3	No	4
(light shading)	3	Yes	4
(dark shading)	24	Yes	6

* PIGPA (SOLUTION A)	
Pyruvate	50 mmoles/L
Inosine	50 "
Glucose	100 "
Phosphate	50 "
Adenine	5 "
NaCl	9 gm/L
pH	7.2
mOs/kg	650

FIGURE 8A

Changes in 2,3 DPG levels of acid-citrate-dextrose (ACD) and citrate-phosphate-dextrose (CPD) red cells during storage at 4 C. Some of the units of red blood cells were stored at 4 C in CPD for 3 to 5 days, incubated with PIGPA (Solution A) at 37 C for 1 hour, and freeze-preserved with 40% W/V at -80 C, or with 20% W/V at -150 C. Other units were stored in ACD or CPD at 4 C for 28 days, incubated with PIGPA (Solution A), and freeze-preserved with 40% W/V at -80 C, or with 20% W/V at -150 C. All of the units were washed before transfusion.

VALERI - FIGURE 8A

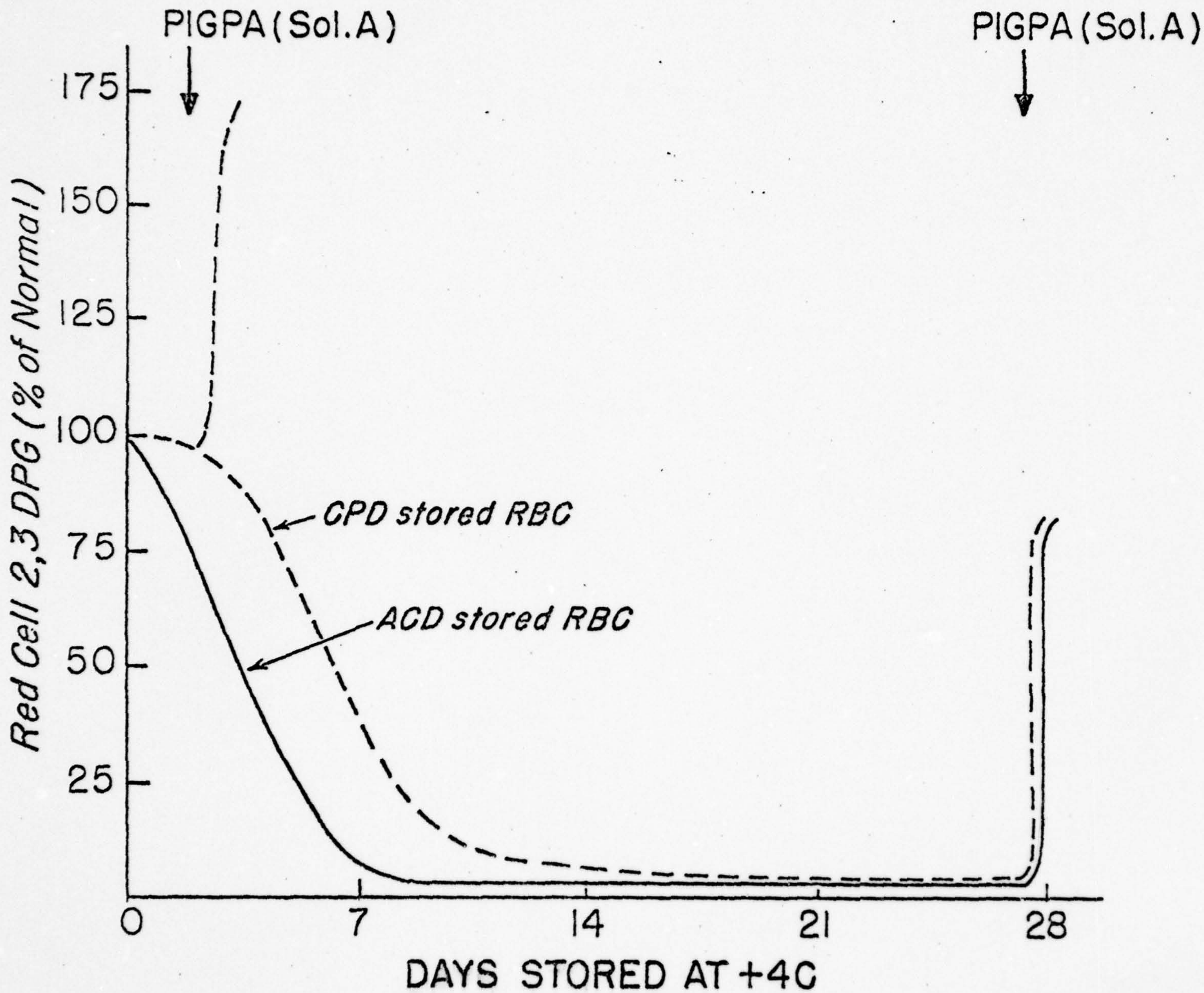


FIGURE 8B

Changes in the ATP levels of acid-citrate-dextrose (ACD) and citrate-phosphate-dextrose (CPD) red cells during storage at 4 C. Some of the units of red blood cells were stored at 4 C in CPD for 3 to 5 days, incubated with PIGPA (Solution A) at 37 C for 1 hour, and freeze-preserved with 40% W/V at -80 C, or with 20% W/V at -150 C. Other units were stored in ACD or CPD at 4 C for 28 days, incubated with PIGPA (Solution A), and freeze-preserved with 40% W/V at -80 C, or with 20% W/V at -150 C. All of the units were washed before transfusion.

VALERI - FIGURE 8B

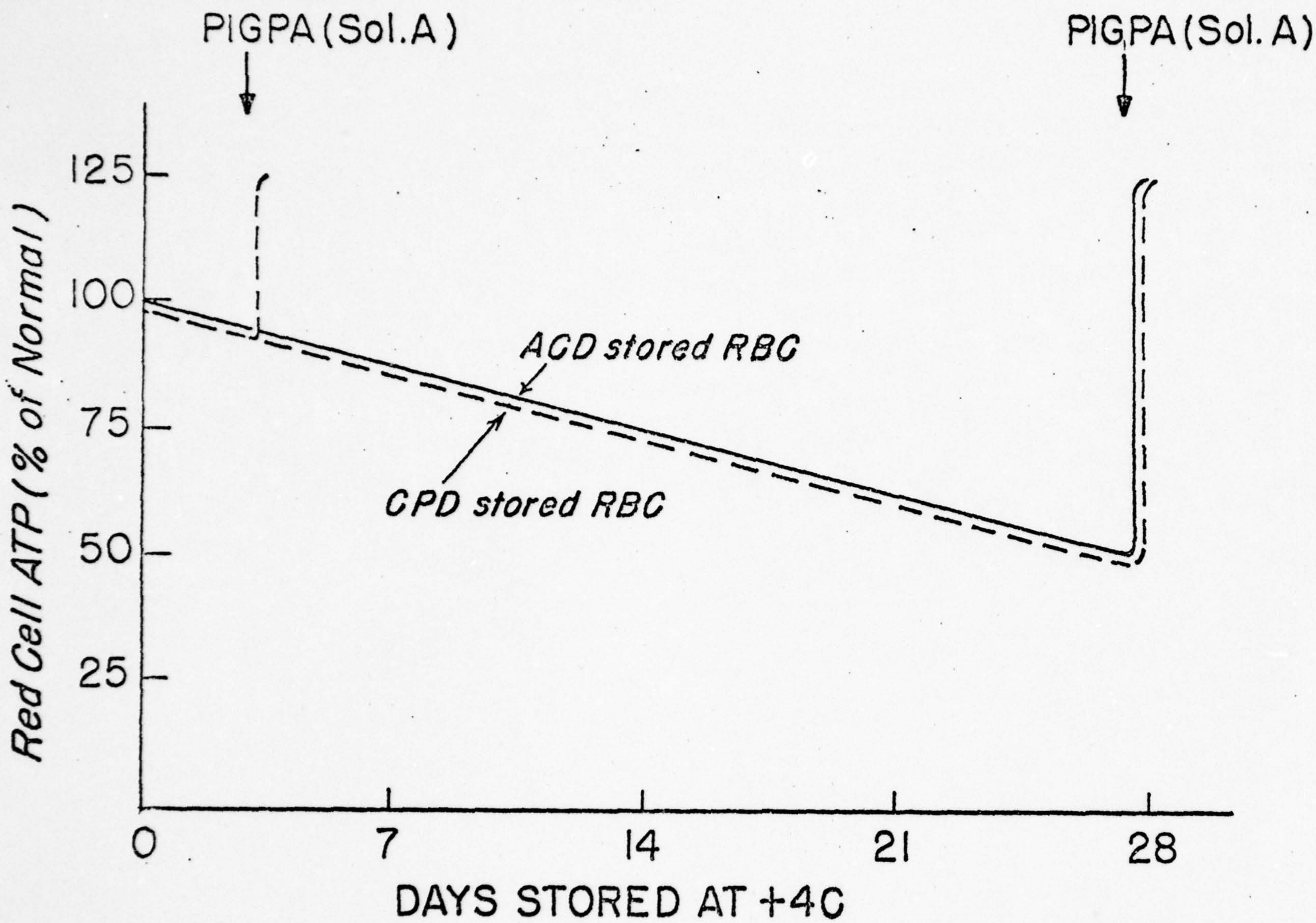
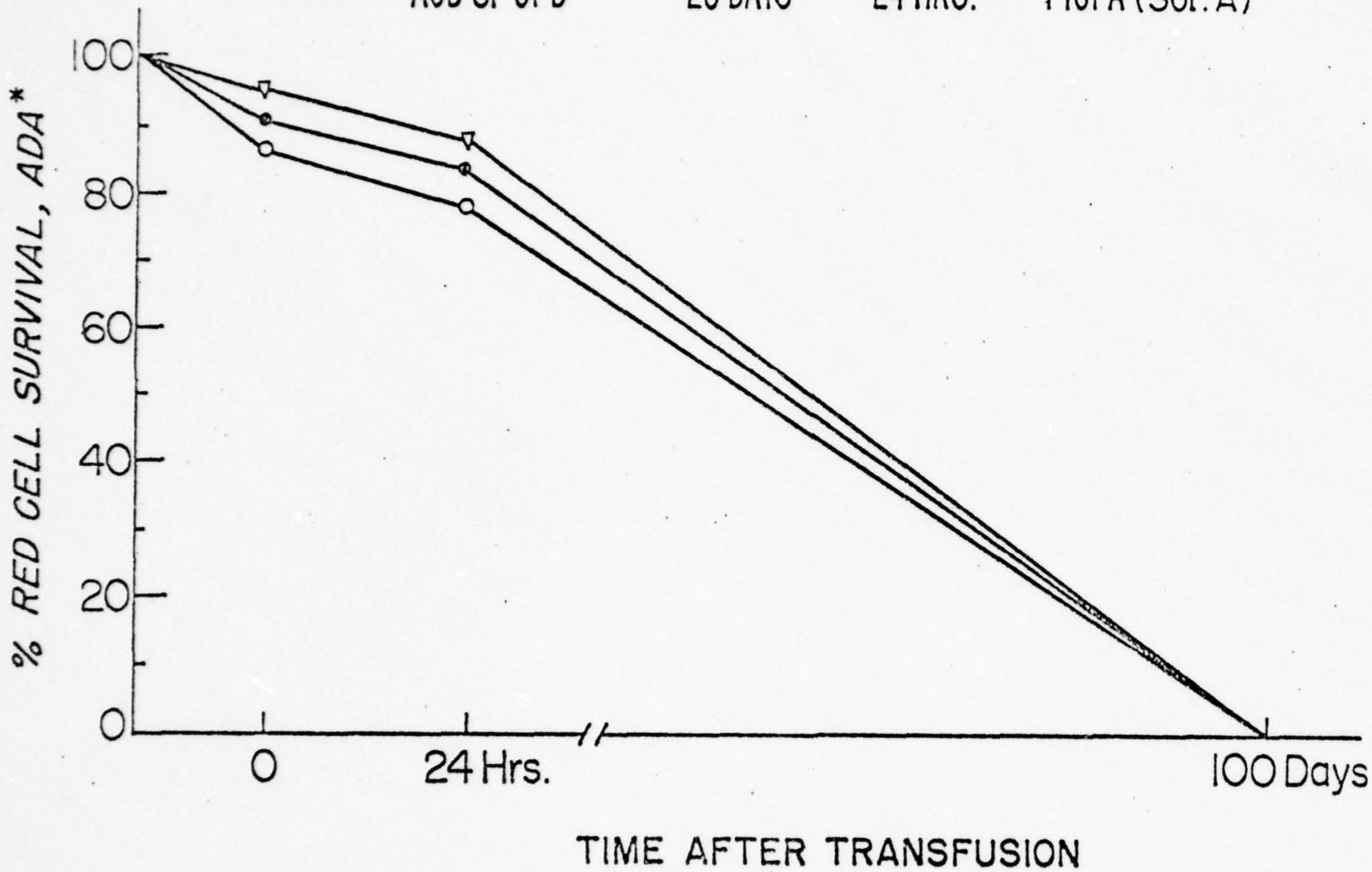


FIGURE 8C

24-Hour posttransfusion survival and lifespan values of: (1) non-rejuvenated red cells--red cells stored at 4 C in ACD or CPD for 3 to 5 days, frozen with 40% W/V glycerol and stored at -80 C, or with 20% W/V glycerol and stored at -150 C, washed, and stored at 4 C in a sodium chloride-glucose-phosphate solution for 24 hours before transfusion; (2) indated-rejuvenated red cells--red cells stored at 4 C in CPD for 3 to 5 days, rejuvenated with PIGPA (Solution A), frozen with 40% W/V glycerol and stored at -80 C, or with 20% W/V glycerol and stored at -150 C, washed, and stored at 4 C in a sodium chloride-glucose-phosphate solution for 24 hours before transfusion; and (3) outdated-rejuvenated red cells--red cells stored at 4 C in ACD or CPD for 28 days, rejuvenated with PIGPA (Solution A), frozen with 40% W/V glycerol and stored at -80 C, or with 20% W/V glycerol and stored at -150 C, washed, and stored at 4 C in a sodium chloride-glucose-phosphate solution for 24 hours before transfusion.

VALERI - FIGURE 8C

	ANTI-COAG.	TIME STORED AT +4C PRE-FREEZE	TIME STORED AT +4C POST-WASH	REJUVENATION MEDIUM
▽—▽	ACD or CPD	3 to 5 DAYS	24 HRS.	—
●—●	CPD	3 to 5 DAYS	24 HRS.	PIGPA (Sol. A)
○—○	ACD or CPD	28 DAYS	24 HRS.	PIGPA (Sol. A)



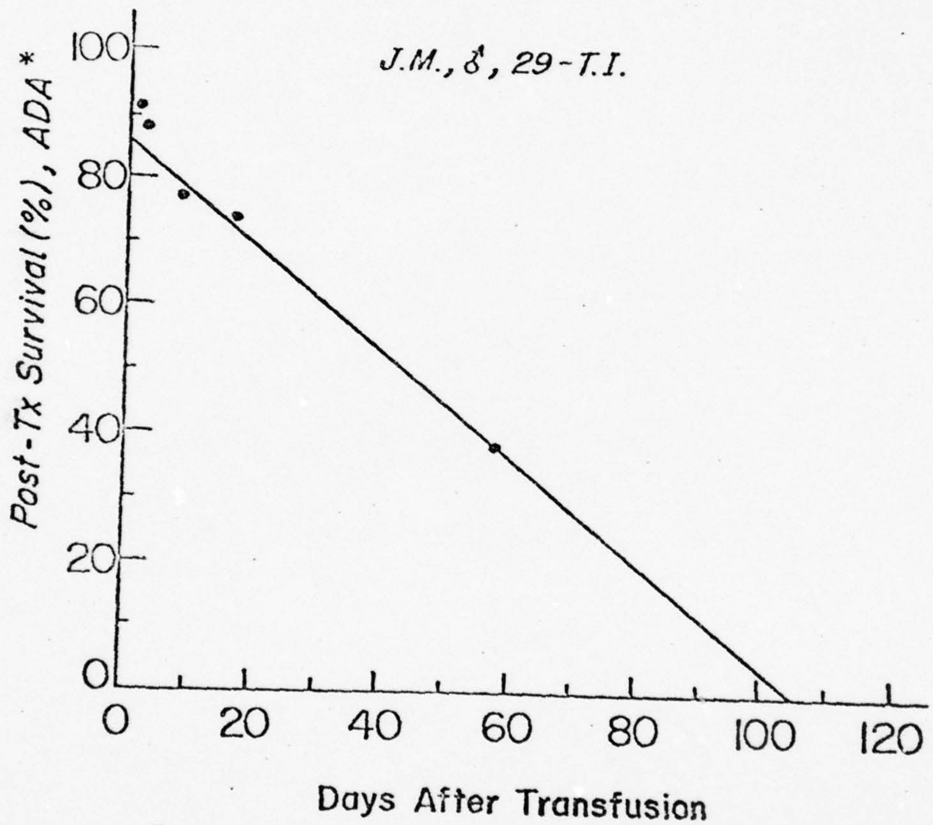
*Automated Differential Agglutination

FIGURE 9A

Posttransfusion survival measured by an automated differential agglutination procedure, and red cell ATP, 2,3 DPG, P_{50}, and pH levels on the day of transfusion. Red cells were stored at 4 C in acid-citrate-dextrose (ACD) for 26 days prior to rejuvenation with PIGPA (Solution A), and freeze-preservation with 40% W/V glycerol in an ionic medium at -80 C for 3 months. After thawing, each unit was washed in the Haemonetics Blood Processor 15 by gravity flow with 3.2 liters of sodium chloride solutions and stored at 4 C in a sodium chloride-glucose-phosphate solution for 48 hours before transfusion. Three units of red cells were transfused to J.M., a 29-year-old male with traumatic injuries.

VALERI - FIGURE 9A

UNITS	ANTI-COAG.	DAYS STORAGE AT +4C PRE-FZ	POST-WASH	REJUVENATION MEDIUM	ATP	2,3-DPG (μM/g.Hb)	p50 (mm Hg)	RED CELL pH(37C)
3	ACD	26	2	PIGPA (Sol.A)	3.3	6.0	28.5	6.19



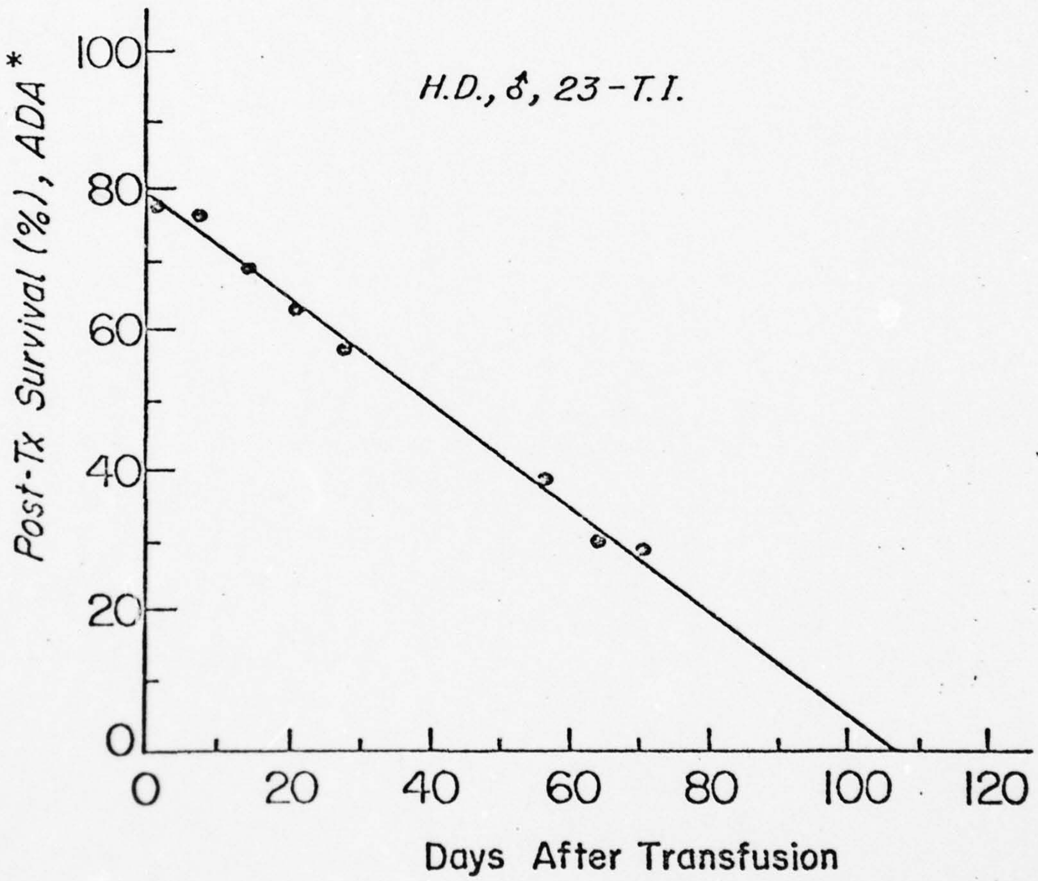
*Automated Differential Agglutination

FIGURE 9B

Posttransfusion survival measured by an automated differential agglutination procedure, and red cell ATP, 2,3 DPG, P_{50}, and pH levels on the day of transfusion. Red cells were stored at 4 C in ACD for 29 days prior to rejuvenation with PIGPA (Solution A), and freeze-preservation with 40% W/V glycerol in an ionic medium at -80 C for 1 year. After thawing, each unit was washed in the Fenwal Elutramatic with 2.7 liters of sodium chloride solutions, and stored at 4 C in a sodium chloride-glucose-phosphate solution at 4 C for 24 hours before transfusion. Four units of red cells were transfused to H.D., a 23-year-old male with traumatic injuries.

VALERI - FIGURE 9B

UNITS	ANTI COAG.	DAYS STORAGE AT +4C PRE-FZ	POST-WASH	REJUVENATION MEDIUM	ATP (μM/g.Hb)	2,3-DPG	p50 (mm Hg)	RED CELL pH(37C)
4	ACD	29	1	PIGPA (Sol. A)	4.3	6.8	26.9	6.32



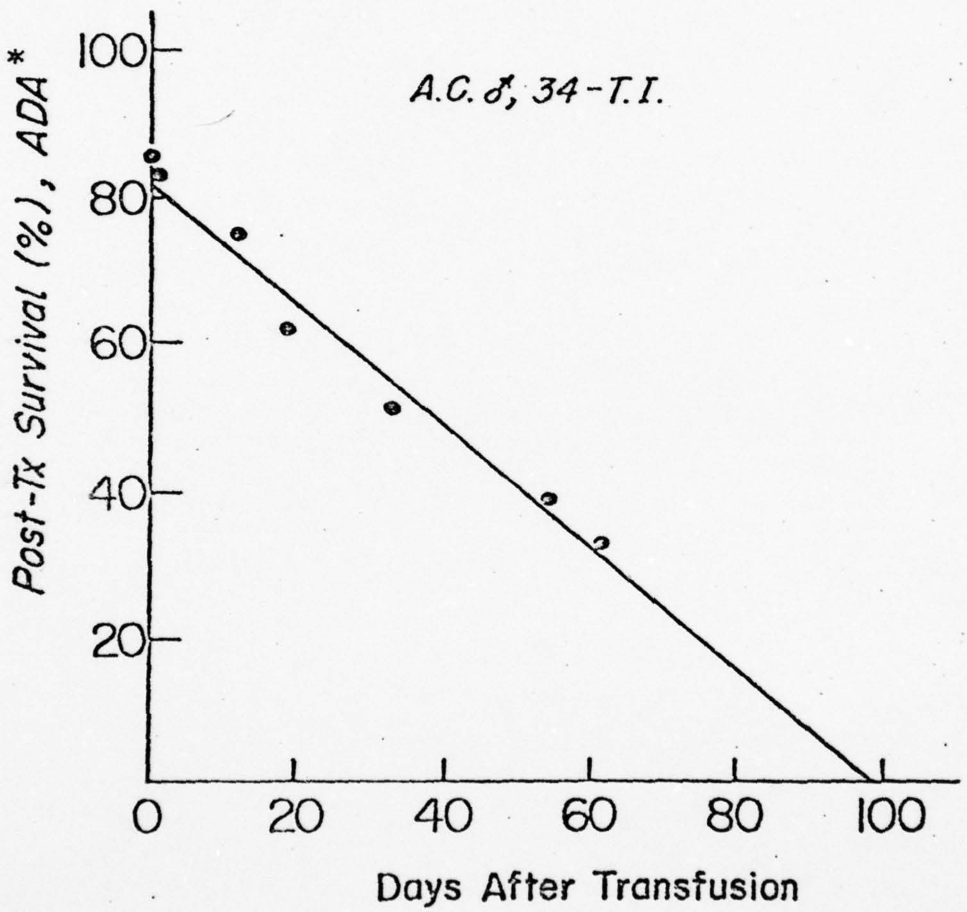
*Automated Differential Agglutination

FIGURE 9C

Posttransfusion survival measured by an automated differential agglutination procedure, and red cell 2,3 DPG, ATP, P_{50}, and pH levels on the day of transfusion. Red cells were stored in CPD at 4 C for 2 days prior to rejuvenation with PIGPA (Solution A), and freeze-preservation with 40% W/V glycerol in an ionic medium at -80 C. They were washed in the IBM Blood Processor with 2.2 liters of sodium chloride solutions, and stored in a sodium chloride-glucose-phosphate solution at 4 C for 24 hours prior to transfusion. Four units of red cells were transfused to A.C., a 34-year-old male with traumatic injuries.

VALERI - FIGURE 9C

UNITS	ANTI-COAG.	DAYS STORAGE AT +4C PRE-FZ	POST-WASH	REJUVENATION MEDIUM	ATP (μM/g.Hb)	2,3-DPG	p50 (mm Hg)	RED CELL pH(37C)
4	CPD	2	1	PIGPA (Sol. A)	3.9	16.5	39.2	6.42



*Automated Differential Agglutination

FIGURE 9D

Posttransfusion survival measured by an automated differential agglutination procedure, and red cell ATP, 2,3 DPG, P_{50}, and pH levels on the day of transfusion are reported for each population. Red cells were freeze-preserved with 40% W/V glycerol at -80 C, and washed in the Haemonetics Blood Processor 10 with 3.2 liters of sodium chloride solutions before transfusion to W.S., a 20-year-old male with traumatic injuries. The 4 units of O-positive red cells had been stored in CPD for 2 days prior to rejuvenation with PIGPA (Solution A) and freeze-preservation. After washing they were stored in a sodium chloride-glucose-phosphate solution at 4 C for 24 hours prior to transfusion. The 2 units of A-negative red cells had been stored in ACD for 32 days prior to rejuvenation with PIGPA (Solution A) and freeze-preservation. After washing they were stored in a sodium chloride-glucose-phosphate solution at 4 C for 24 hours prior to transfusion.

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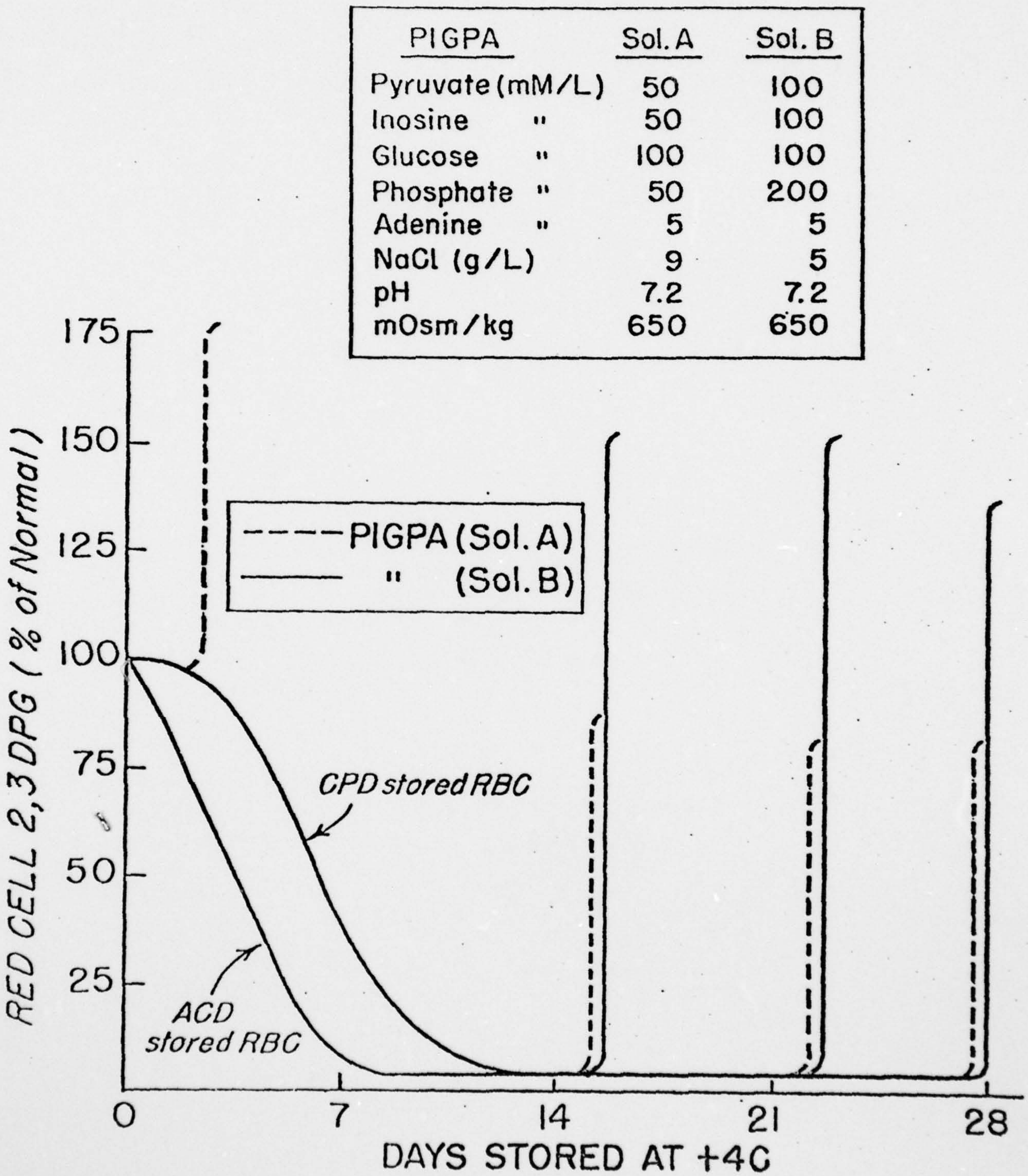
FIGURE 10A

The 2,3 DPG levels in red cells during storage in ACD or CPD for up to 28 days at 4 C. The red cells were biochemically modified by incubation for 1 hour with PIGPA Solution A or Solution B in some units after 3 to 5 days of storage, in some units after 14 to 16 days of storage, and in some units after 22 to 28 days of storage. The biochemically modified units were concentrated and freeze-preserved by the high or low glycerol method.

VALERI - FIGURE 10A



PIGPA	Sol. A	Sol. B
Pyruvate (mM/L)	50	100
Inosine "	50	100
Glucose "	100	100
Phosphate "	50	200
Adenine "	5	5
NaCl (g/L)	9	5
pH	7.2	7.2
mOsm/kg	650	650

FIGURE 10B

The ATP levels in red cells during storage in ACD or CPD for up to 28 days at 4 C. The red cells were biochemically modified by incubation for 1 hour with PIGPA Solution A or Solution B in some units after 3 to 5 days of storage, in some units after 14 to 16 days of storage, and in some units after 22 to 28 days of storage. The biochemically modified units were concentrated and freeze-preserved by the high or low glycerol method.

VALERI - FIGURE 10B

PIGPA	Sol. A	Sol. B
Pyruvate (mM/L)	50	100
Inosine "	50	100
Glucose "	100	100
Phosphate "	50	200
Adenine "	5	5
NaCL (g/L)	9	5
pH	7.2	7.2
mOsm/kg	650	650

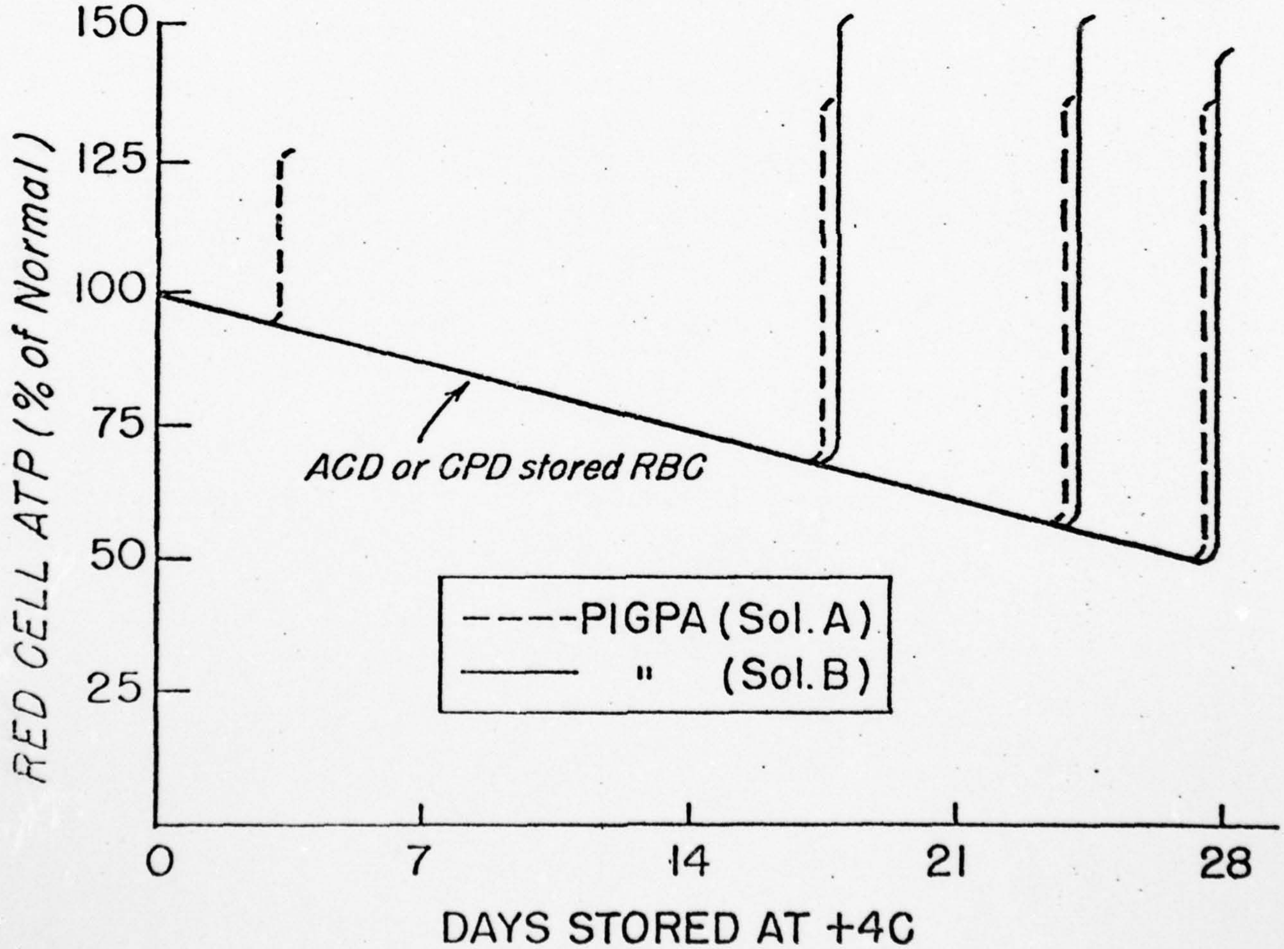
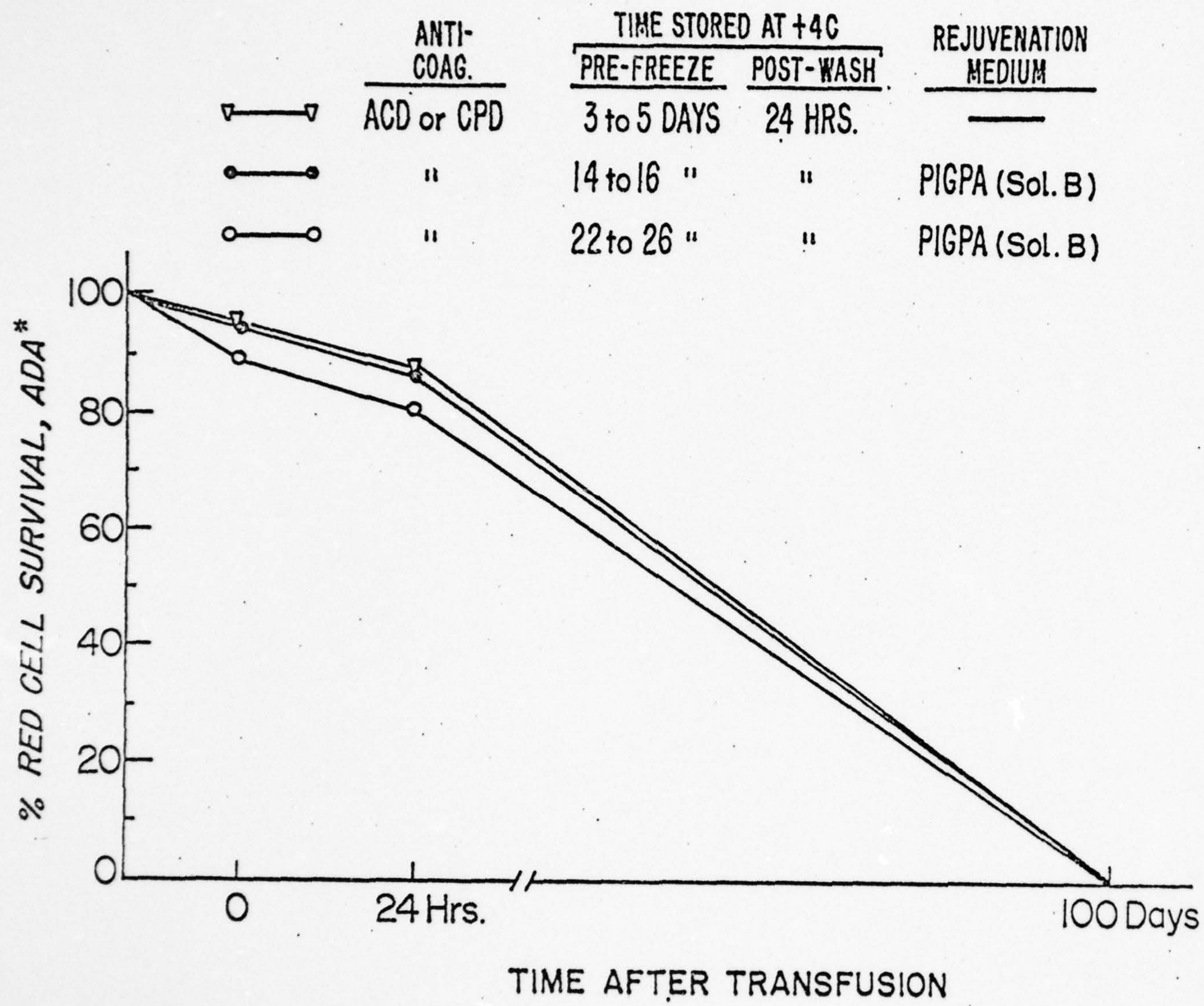


FIGURE 10C

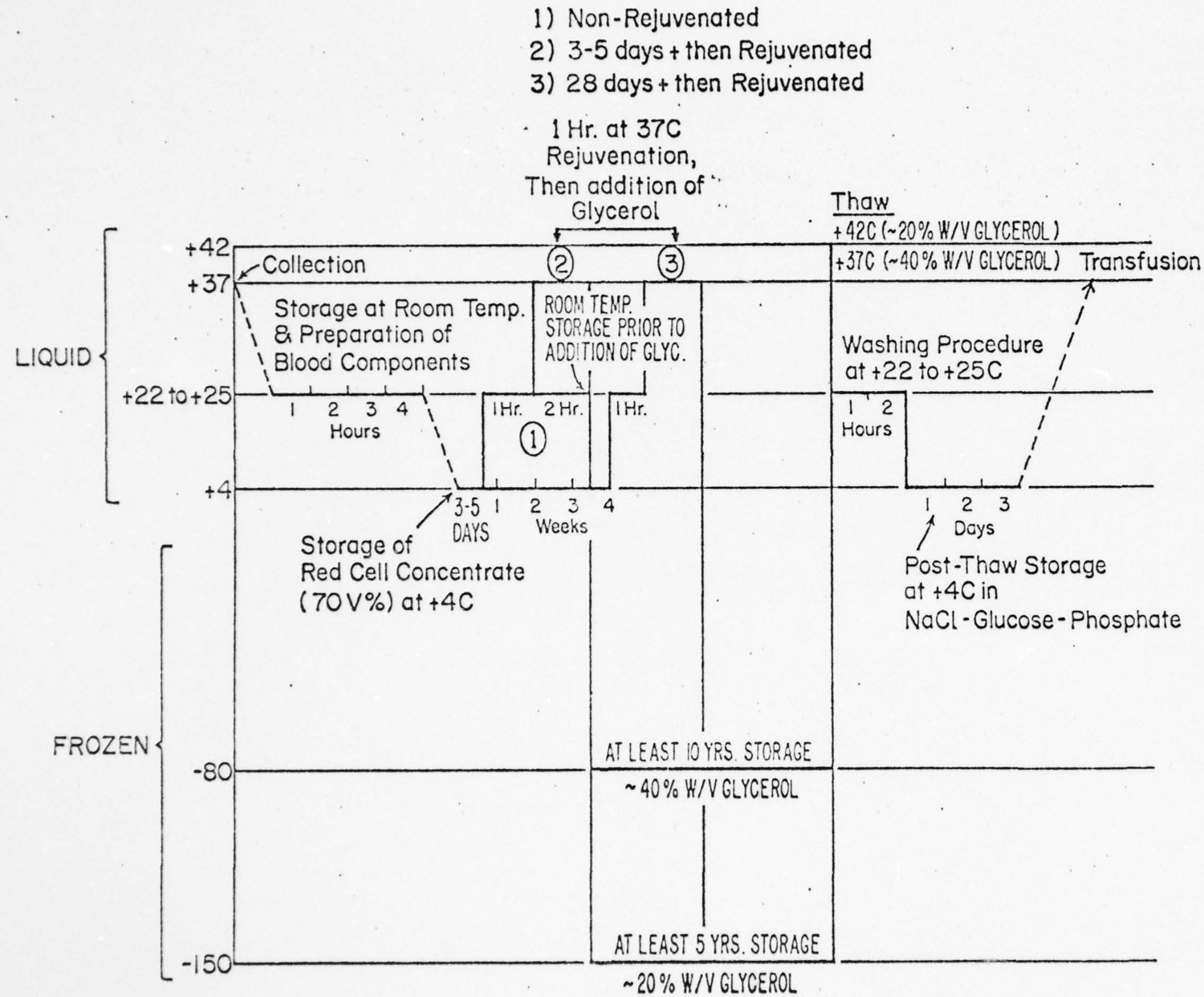
24-hour posttransfusion survival and lifespan of non-rejuvenated and rejuvenated red cells after freeze-preservation with high (40% W/V) or low (20% W/V) concentration of glycerol, washing, and storage in sodium chloride-glucose-phosphate at 4 C for 24 hours. Non-rejuvenated red cells are those that were stored in ACD or CPD at 4 C for 3 to 5 days. The rejuvenated red cells are those that were stored at 4 C in ACD or CPD for 14 to 16 days or for 22 to 26 days before biochemical modification with PIGPA Solution B.



* *Automated Differential Agglutination*

FIGURE 11

Storage temperatures of human red cells during liquid and freeze-preservation.



1) Non-Rejuvenated
2) 3-5 days + then Rejuvenated
3) 28 days + then Rejuvenated
1 Hr. at 37C Rejuvenation, Then addition of Glycerol
Thaw
+42C (~20% W/V GLYCEROL)
+37C (~40% W/V GLYCEROL)
Transfusion
LIQUID
FROZEN
+42
+37
+22 to +25
+4
-80
-150
Collection
Storage at Room Temp. & Preparation of Blood Components
1 2 3 4
Hours
ROOM TEMP. STORAGE PRIOR TO ADDITION OF GLYC.
1 Hr.
2 Hr.
1 Hr.
3-5 DAYS
1 2 3 4
Weeks
Storage of Red Cell Concentrate (70 V%) at +4C
Washing Procedure at +22 to +25C
1 2
Hours
1 2 3
Days
Post-Thaw Storage at +4C in NaCl - Glucose - Phosphate
AT LEAST 10 YRS. STORAGE
~40% W/V GLYCEROL
AT LEAST 5 YRS. STORAGE
~20% W/V GLYCEROL

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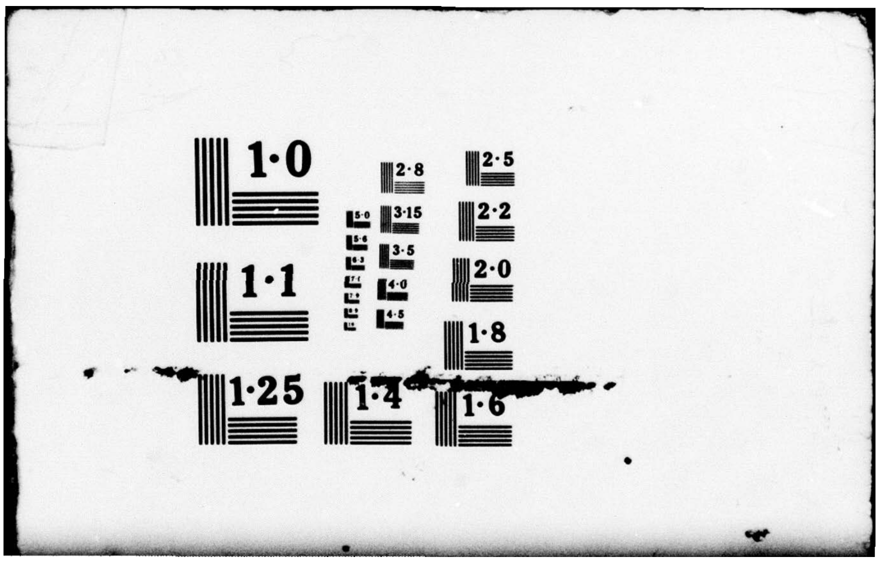
1·0
2·8
2·5
5·0
3·15
2·2
5·6
3·5
1·1
2·0
4·0
4·5
1·8
1·25
1·4
1·6

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ABSTRACT

An anemic pregnant black primagravida with hemoglobin SD studied throughout pregnancy and parturition. Historically, the patient appeared to be Sickle Cell Anemic but the fact that she was pregnant suggested a variant hemoglobinopathy. Hemoglobin SD disease was established by agar gel electrophoresis at pH 6.45. This patient carried to term a healthy female infant which would be unusual in a patient with homozygous hemoglobin S disease. An increase in the red cell concentration of 2,3 diphosphoglycerate (2,3 DPG) (range 18-21 uM/g Hb; normal 12±2 mm Hg) was associated with a decrease in red cell oxygen affinity. Red cell oxygen affinity was measured by the *in vitro* P50 value of the oxyhemoglobin dissociation curve, the P50 value is equal to the oxygen tension at 50% saturation of hemoglobin. The patient's *in vitro* P50 value was found to a range between 35-45 mm Hg (normal 28±1 mm Hg). An elevation in red cell 2,3 DPG is usually associated with decreased red cell oxygen affinity and the functional role of

of this red cell adaptation (elevated RBC 2,3 DPG and decreased RBC oxygen affinity) in a pregnant woman with hemoglobin SD disease is discussed.

RESULTS:

Moderately severe anemia with hemoglobin values less than 9 g/dl and microhematocrit values less than 25 volumes % were recorded throughout gestation. The patient's anemia was associated with a marked reticulocytosis, greater than 23% (Table I).

Hemoglobin electrophoresis (by starch gel electrophoresis at pH 8.5 and agar gel electrophoresis at pH 6.2) indicates 32% hemoglobin S and 58% hemoglobin D (Table I).

At three days following delivery, the patient's ^{51}Chromium red cell volume was decreased by 280 ml and her 125Iodine-labeled albumin plasma volume was expanded by 1810 ml when compared to her theoretical values (Table I).

Red blood cell 2,3 DPG was 155% of normal throughout gestation and fell toward normal shortly after delivery but began to rise again during parturition (Figure I). Associated with the increased red blood cell 2,3 DPG is an elevated in vitro P50 value, 150% of normal, throughout the gestation and parturition

(Figure I). The in vitro P50 value did not ever approach the normal range (Figure I).